JOURNAL OF AGRICULTURAL RESEARCH

VOL. XII WASHINGTON, D. C., JANUARY 14, 1918 NO. 2

PURE CULTURES OF WOOD-ROTTING FUNGI ON ARTIFICIAL MEDIA

By W. H. LONG, *Forest Pathologist*, and R. M. HARSCH, *Assistant in Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture*[1]

INTRODUCTION

The study of wood-rotting fungi by means of cultures on artificial media has been very meager in the past compared to the almost universal use of cultural methods by bacteriologists and workers with strictly parasitic fungi. A critical study of the existing literature on cultures of wood-rotting fungi develops the fact that much of this work was either not done under proper control conditions where the purity of the organism under investigation was guaranteed or the media used by many of the workers consisted of pieces of wood, bread, dung decoctions, etc., and not artificial media of such a character that others could reproduce the media, growth conditions, etc., and thus repeat and verify the experiments.

Brefeld (*1-3*)[2], Falck (*5-7*), Humphrey and Fleming (*8*), Lyman (*11*), Rumbold (*12*), and Zeller (*13, 14*) are some of the workers who have made cultural studies of a number of hymenomycetous fungi on a rather extensive scale. However, the line of investigation followed by most of them has been more along the lines of polymorphism in spore forms, enzymic action, the rot caused by each fungus, and the prevention or control of these fungi in the rotting of structural timber rather than a critical study of their cultural characters on artificial media. Probably the most serious drawback to investigators in working with wood-rotting fungi, especially the Polyporaceae, has been the fact that it was not possible under conditions used by them to obtain with any degree of certainty the sporophores of the various fungi on artificial media.

This paper deals with two lines of investigation of fungous activity when grown in pure cultures: (1) A method by which various wood-rotting fungi can be differentiated from each other by their cultural characters alone when grown upon artificial media; and (2) a method by which the

[1] The writers are under obligations to Dr. E. A. Burt for assistance in identifying the various species of the Thelephoraceae, and to Dr. W. A. Murrill for identifying the more difficult species of the Polyporaceae discussed in this paper.

[2] Reference is made by number (italic) to "Literature cited," pp. 81–82.

fruiting bodies or sporophores of wood-rotting fungi can be produced from pure cultures on artificial media. In this paper the writers have only given in a general way the results of somewhat extended investigations on many species of wood-rotting fungi, leaving for a later article a large amount of detail and the discussion of special methods and culture media which they have found very valuable in working with this group of organisms.

GENERAL METHODS OF EXPERIMENTATION

ORIGIN OF CULTURES

The initial cultures of all of the wood-rotting fungi under investigation by the writers have been obtained from the three following sources: (1) Small pieces of diseased wood, (2) small pieces of sporophores, and (3) spores. Pieces of inoculum 4 or 5 mm. in diameter have been found to be better than smaller pieces. The old idea that the smaller the piece the freer it is from contamination is good reasoning theoretically, but in actual practice pieces of the size mentioned above have been found more viable than small bits and as free from contamination. The larger the piece the greater are the chances of viable mycelium being present. The pieces should be inserted endwise into the middle of the agar slant until about one-half of the wood is buried in the agar. Care should be taken to avoid, as far as possible, burying the wood in the agar, since the covering of agar excludes the air and either retards or prevents entirely the fungus from starting on the culture medium. A pair of long-handled scissors or forceps are especially suitable for this work.

In initial cultures the writers have found it very convenient to use a series of 10 tubes, including 2 tubes each of carrot, malt, cornmeal, prune, and parsnip agars. These agars have been found to give a fairly good growth of mycelium, and at the same time indications of the fruiting, cultural characters, etc. of the organism may be obtained even from these initial cultures.

The writers have made approximately 10,000 cultures of wood-rotting fungi in their preliminary studies here reported. All of the inoculations, both initial and subcultures, have been made in an open room without the use of any special inoculating chamber. The percentage of pure subcultures obtained when the original tube was uncontaminated has been very high. For instance, out of 1,000 transfers recently made only 7 contaminated tubes were found.

METHODS USED IN MAKING SUBCULTURES

The writers desire to describe here a method which they have found very useful in making transfers of fungus cultures when 10 or more transfers are to be made from the same tube. The instruments used in these transfers are a pair of long-handled scissors made by lengthening the handles of a pair of dissecting scissors, a small square glass jar with a

triangular section cut out of the aluminum screw top large enough to hold the plug from the mother tube, and a salt-mouth bottle (holding 300 or 400 c. c.) filled about two-thirds full with 95 per cent alcohol. The blades of the scissors used in making the transfers are kept in this 95 per cent alcohol when not in actual use. The glass jar and top are cleaned by washing in hot water and then dried before using. The opening in the top is thoroughly flamed over an alcohol lamp or Bunsen burner and the jar is placed on its side with the triangular opening toward the operator. During the actual inoculation the cotton plug from the mother tube is placed in the triangular opening with the lower end of the plug inside the jar in such a manner that only the sharp edges of the top of the jar come in contact with the cotton plug. In this position the plug is protected from outside contamination and at the same time the hands of the operator are left free to handle the scissors, two culture tubes, and the cotton plug from the tube to which the transfer is being made.

In making the transfers of certain standardized series it was necessary to obtain small inocula as near the same size for each transfer as possible. The ordinary inoculating needles and loops made either of platinum or of iridio-platinum are too soft and in other ways unsuited for making transfers of fungus mycelium. The writers therefore adopted the use of the scissors for such work, since by using them the mycelial layer on the surface of the agar in the culture tubes can be readily cut and any desired size of inoculum transferred without loss of time and with a minimum of outside contamination.

VEGETATIVE CULTURAL CHARACTERS ON ARTIFICIAL MEDIA

MEDIA USED

In studying the cultural characters of the various fungi as outlined under No. 1 of the introduction, the following general system was adopted: A series of 10 different culture media in agar was used for each fungus. These 10 media were (1) 1.5 and 2 per cent carrot agar, +3.5 to +5.0; (2) 1.5 and 2 per cent malt agar, +7.0; (3) 1.5 per cent beet agar, +2.5 and +3.0; (4) 1.5 per cent celery agar, +9.5 to +15.5; (5) 1.5 per cent bean agar, +1.0 to +1.5; (6) 1.5 and 2 per cent corn-meal agar, +0.25; (7) 1.5 and 2 per cent prune agar, +1.0 and +1.5; (8) 1.5 and 2 per cent alfalfa agar, +13.5 to +15.5; (9) 1.5 per cent parsnip agar, +9.0 to +13.5; and (10) 1.5 and 2 per cent potato agar, +2.0 and +3.5. The acidity of the media here given is based on Fuller's scale and is the actual acidity of the media after tubing and as used in the cultures.

In any series of a given fungus each corresponding agar for each strain had the same percentage and the same acidity. For instance, there were nine strains of *Trametes pini* compared. The carrot agar used for each of these nine sets was 2 per cent and had an acidity of +3.5.

The writers selected the 10 media for the study of the cultural characters of the different fungi not with a view to obtaining vigorous growth but

to get media on which the growth on each would be different for the same organism. In other words, the media used were not intended to develop general characters but specific ones which might differentiate the fungus under investigation from other closely related species.

NUMBER OF TUBES OF EACH MEDIUM INOCULATED

One tube of each of these 10 media was used in the series for any given fungus. Better results would probably have been obtained by using three or more tubes of each medium rather than one, but the writers could not do this in their preliminary work for lack of sufficient equipment. However, in a great majority of cases it is believed that accurate results were obtained with these series of 10, since many of them have been repeated to the fourth and fifth subcultures with different batches of media, and the resulting characters when grown under the conditions described below were practically identical for each subculture of the fungus for each medium.

POSITION OF CULTURE TUBES IN REFERENCE TO GRAVITY

After inoculating the series of 10 tubes, they were placed in a horizontal position, side by side, in shallow boxes with the surface of the agar slant uppermost. The boxes were from 2 to 4 cm. deep and about 14 to 14.5 cm. wide. The culture tubes (150 mm. long or longer) had their tops resting on the upper edges of the boxes and were therefore tilted at a slight angle. These boxes were then placed on shelves in front of windows with a western exposure where they received all of the diffused light which came through and during the afternoon received the direct rays of the sun from one to four hours daily. Under these conditions the agar in the tubes gradually dried and the upper portion of it separated from the glass, leaving a space of varying depth between the agar and the glass, on which the aerial mycelium could grow even to the bottom of the tube.

AMOUNT OF DIRECT SUNLIGHT CULTURES RECEIVED

The earlier cultures of the writers received only one or two hours of direct sunlight. As the season advanced, the quantity of direct sunlight received became greater, until finally the amount received was judged to be too great and the intensity of the direct sunlight was decreased, first, by a single screen of cheesecloth tacked over the front of the frames holding the culture tubes. Later, a second piece of cheesecloth was tacked over the first one. The general effect of the sunlight on the cultures in the tubes thus exposed was to check the growth of the fungi, compared to similar tubes when placed in very weak, diffused light or absolute darkness. The sunlight also seems to intensify the colors of the aerial mycelium when it is normally other than white.

TEMPERATURE RECORDS

During the entire time the cultures were under observation, two thermographs were run continuously. One was placed on the shelf with the fungi exposed to sunlight. A soil thermograph was used to record the temperature of the tubes kept in the dark.

DEFINITION OF TERMS USED

It was found early in the study of the cultural characters of the fungi under consideration that a set of descriptive terms especially adapted to the cultures of fungi grown under the conditions here described would have to be used. The terms employed in the tables in this paper and in the body of the text are those usually found in ordinary botanical literature, but they have been modified somewhat to fit the conditions obtaining for fungus growth. The fungus growth on artificial media is divided by the writers into two general classes, aerial and submerged. The aerial mycelium consists of that which is on or above the surface of the agar; the submerged mycelium includes all that is beneath the surface of the agar.

Great difficulty was found in obtaining appropriate terms which would express the character of growth of the aerial mycelium. In describing this aerial growth terms which are usually used in describing the pubescence of leaf surfaces have been employed. In other words, the surface of the agar is considered as the surface of a leaf and the character of the mycelium growing on this surface is discussed in terms of leaf pubescence with some minor modifications made necessary by the character of the organism under discussion. The following terms as used by the writers require special definition, since they depart in some instances from the usually accepted definitions of these terms:

Appressed: Mycelium which is prostrate on the surface of the agar. This with many fungi is the first stage in the aerial growth of the mycelium. Later this appressed mycelium may give place to other forms.

Cobwebby: Long, weak, intertangled hairs which are not thick enough to be either woolly or felty and are not short enough to be considered as downy.

Cottony: Erect, rather long (3 to 5 mm.) mycelium spreading in all directions.

Downy: Short, fine hairs, loosely scattered over the surface of the mycelium, giving it a downy appearance.

Felty: Matted with intertwined hairs, resembling felt.

Floccose: Scattered patches of short mycelium.

Plumose: Tufts of mycelium with a central axis from which short hyphae radiate.

Silky: Long parallel threads of mycelium, more or less prostrate, like combed silk.

Sodden: Mycelium having a water-soaked appearance; usually such mycelium is appressed.

Subfelty: A thin layer of mycelium consisting of short intertwined hairs.

Velvety: Layer of mycelium with distinct, dense, straight, short hairs like pile of velvet.

Woolly: A dense mass of mycelium consisting of long, tortuous, matted hairs. Cottony and woolly may both later become felty by the long hairs becoming matted and prostrate.

Hyphenated compound words, like "appressed-downy," "felty-woolly," indicate a condition intermediate between the two names, while "downy to appressed" means that the older portions are downy, while the younger portions are appressed.

In all of the tubes the growth of the fungus is both lateral and longitudinal. Of course, the lateral extension is very limited, since the inside diameter of the tubes is only about 20 mm., while the length varies with the length of the agar in the tube. The first record for growth shown in all the tables is always that of the lateral growth. For instance, a record showing 20 by 30 mm. means that the lateral growth was 20 mm. and the longitudinal 30 mm. When the same set of figures are repeated for two intervals of time, like 60 days, 20 by 80 mm., and 80 days, 20 by 80 mm., this indicates that the growth of the fungus had reached the bottom of the tube at the first record given and would therefore be the same for the second interval of time.

IMPORTANT DIFFERENTIAL CRITERIA

The following criteria have been found of value in the differentiation of the various species: (1) Macroscopic characters, including rapidity of growth, color of aerial and submerged mycelium, character of aerial mycelium such as to texture, etc., staining of the agar, decoloration of the agar, the comparative rate of growth between the aerial and submerged mycelium, especially when the submerged mycelium is colored and markedly in advance of the aerial; (2) microscopic characters, such as septation, branching, size and color of hyphæ, clamp connections, polymorphism in spore formation, etc. A few of the species of Polyporaceae examined by the writers have in addition to the usual basidiospores other spore forms variously known as conidia, oidia, chlamydospores, etc. These various nonbasidiosporic forms may be divided into two general groups, spores which are borne on the aerial hyphae and the so-called spores which are borne on the submerged hyphae, often referred to as chlamydospores. The latter have been found by the writers to be more widely distributed in the Polyporaceae than the aerial spores and their presence and characters as well as those of the aerial should always be noted, since they are of great diagnostic value.

It will be noted from the tables that certain fungi have colorless or white aerial mycelium throughout on certain agars, while others have colored depending upon the agar used. Such color differences are very important, since they are usually constant for a given species on a given agar.

In some species of the fungi examined the submerged mycelium in certain media is constantly colored, while in other species, whether the submerged mycelium is colored or colorless, seems to depend upon certain environmental factors, such as the amount of moisture present in the medium or the acidity or alkalinity of the medium.

Some of the most important criteria for distinguishing different but closely related fungi are found in the first 10 or 15 days of the growth of the subcultures, such as rapidity of growth, color changes in the mycelium, staining of the agar, decoloration of media, etc. Important characters which are sharply defined at one stage of growth often disappear or are obscured by the later mycelial development; and for this reason the cultural data in the tables have been given for several periods of time in the growth of the cultures, say at 10, 20, and 30 day intervals.

INFLUENCE OF SUNLIGHT ON CULTURAL CHARACTERS

One of the special benefits which seems to be derived from exposing cultures to the sunlight is the accentuating of the color characteristics and toning down of the mycelial growth of the fungus, thereby making it more characteristic and uniform for a given species than when placed under similar conditions in the darkness.

The differentiation of the characters of the mycelium produced, both as to texture and color of the aerial mycelium, is very much more marked when the cultures are grown in the presence of light at ordinary room temperatures than when grown in incubators at the optimum and constant temperature for the mycelial growth of the fungus under consideration. This probably explains why no one up to the present time has seriously attempted to differentiate the various species of wood-rotting fungi by means of cultural characteristics alone.

Furthermore, the cultures when grown in darkness and at a more or less constant and high temperature overrun very rapidly the surface of the agar in the tube, thus obscuring the real growth of the fungus as observed in the cultures subject to daylight conditions.

GROWTH OF WOOD-ROTTING FUNGI ON AGARS

TEXTURE.—In the growth of wood-rotting fungi on agars the fungus as it spreads from the inoculum on to the surface of the slant proper assumes certain well-defined stages in its growth, which may be roughly divided into two general divisions: (1) Fungi whose advancing young mycelial zone is appressed and (2) fungi whose advancing zone is downy, felty, woolly, etc. There is but little real difference between these two methods of growth, since as a rule the character of the mycelium first to appear is appressed. If the true aerial mycelium, in contradistinction to that which is strictly prostrate on the surface of the agar keeps pace in its growth with the appressed mycelium, the zone of growth will be downy, felty, woolly, etc. If, on the other hand, the growth of the strictly aerial mycelium is much retarded, the appressed mycelium will present a well-defined zone from one to several millimeters across.

The appressed mycelium is usually either colorless or both colorless and sodden, and from this the true aerial mycelium usually develops. The cottony mycelium as a rule does not persist in this condition for

any great length of time. The long, divergent, aerial strands usually become more or less compact and finally felty. The usual steps in the growth of the mycelium of a fungus are first appressed, then downy, then felty, woolly, etc. Cottony mycelium usually develops the cottony stage immediately from the appressed condition. Many of the fungi pass so rapidly from the downy to the felty or woolly stage that it is unnecessary in the description to indicate that there is an intermediate downy stage.

COLORS.—The colors of the fungus as a rule follow certain definite changes. Excluding the color of the mycelium on the inoculum, the first color which usually appears in the early stages of the fungus will be either colorless or white, depending to a considerable extent on whether the young mycelium is appressed or downy. The next step in the color changes will be for the older whitish areas to become light buff[1], warm buff, antimony yellow, etc., if the fungus happens to belong to some of the brown polyporaceae. As the culture ages, the color of the mycelium on the older areas will assume a deeper and deeper tone until finally a color is reached beyond which no appreciable change is observed. In the large majority of cases the color of the older mycelium constitutes a rather extended area compared to that of the younger zone. In practically every instance the cultures obtained from any given fungus on at least several of the culture media will approach very closely the color of the sporophores as they appear in nature. For instance, if one is attempting to grow cultures of *Polyporus dryophilus*, *Fomes texanus*, or other brown fungi, one would expect to have at least several of the culture tubes with brown mycelium similar to that of the fungus.

TABLES SHOWING CULTURAL CHARACTERS

In this preliminary report only a few tables are given out of a large number which the writers have complied on the cultural characters of certain species of wood-rotting fungi. The following species having brown sporophores are given to illustrate the close resemblance in colors, texture, etc., of the same fungus on different hosts: Four strains of *Fomes texanus* (Tables I–IV) and two strains of *Polyporus farlowii* (Tables V–VI).

[1] The colors used in this paper are according to the following standards:
RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

TABLE I.—*Cultural data for Fomes texanus from Juniperus monosperma (FP 21653), New Mexico.*

[Subculture 4 from mycelium on corn-meal agar; initial culture from tissue. Series inoculated on Mar. 12, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	11 days.	22 days.	38 days.	60 days.	11 days.	22 days.	38 days.	60 days.
Carrot		Ochraceous tawny	Argus brown to antimony yellowl.	Argus brown to buckthorn brown.		Brownish	Blackish brown	Blackish brown.
Malt	Warm buff	Antimony yellow.	Amber brown to antimony yellow.	Buckthorn brown to antimony yellow.	Colorless	Chestnut	Liver brown	Do.
Beet		Cinnamon brown to antimony yellow.	Chestnut	Chestnut	do	Blackish brown	Blackish brown	Blackish brown.
Celery		Buckthorn brown to colorless.	Argus brown to sodden.	Argus brown to sodden.		Auburn	do	Do.
Bean	Colorless	Colorless	Colorless	Colorless	Colorless	Russet	Chestnut	Chestnut.
Corn meal	Russet to colorless.	Russet to colorless.	Chestnut to colorless.	Amber to colorless.	Russet	Mummy brown	Liver brown	Mars brown.
Prune	Warm buff	Cinnamon brown to yellow ocher.	Chestnut	Chestnut to amber brown.	Colorless	do	Chestnut	Blackish brown.
Alfalfa	Buckthorn brown.	Sudan brown	Argus brown to buckthorn brown.	Argus brown	do	Prout's brown	Russet	
Parsnip		Russet to cinnamon brown.	Amber brown to antimony yellow.	Chestnut		Mummy brown	Blackish brown	Blackish brown.
Potato	Light buff	Ochraceous tawny to antimony yellow.	Sayal brown	Sayal brown	Colorless	do	Cinnamon brown	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	11 days.	22 days.	38 days.	60 days.	11 days.	22 days.	38 days.	60 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot		Velvety	Velvety	Velvety		20×22	20×30	20×45
Malt	Velvety	do	do	do	4×6	20×25	20×55	20×70
Beet		do	do	do		15×28	18×35	20×45
Celery		Velvety to appressed	Velvety to appressed	Velvety to appressed		15×32	20×35	20×42
Bean	Appressed	Appressed	Appressed	Appressed	4×1	10×12	20×25	20×35
Corn meal	Velvety to appresed	Velvety to appressed	Velvety to appressed	Velvety to appressed	2×3	18×20	20×40	20×60
Prune	Velvety	Velvety	Velvety	Velvety	2×3	20×25	20×40	20×65
Alfalfa	do	do	do	do	12×14	20×35	20×70	20×70
Parsnip		do	do	do		15×25	20×32	20×45
Potato	Velvety	do	do	do	1×2	15×18	15×20	20×30

TABLE II.—*Cultural data for Fomes texanus from Juniperus monosperma (FP 21807), New Mexico.*

[Subculture 2 from mycelium on alfalfa agar; initial culture from tissue. Series inoculated on Mar. 12, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	11 days.	23 days.	38 days.	60 days.	11 days.	23 days.	38 days.	60 days.
Carrot		Ochraceous tawny	Argus brown	Argus brown		Brownish	Liver brown	Blackish brown.
Malt	Warm buff	Buckthorn brown to antimony yellow.	Argus brown to buckthorn brown.	Chestnut to buckthorn brown to antimony yellow.	Colorless	Mummy brown	do	Chestnut.
Beet		Cinnamon brown to antimony yellow.	Chestnut to sodden.	Chestnut	do	Blackish brown	Blackish brown	Blackish brown.
Celery		do	Chestnut	Argus brown to sodden.	do	Mummy brown	do	Do.
Bean	Colorless	Colorless	Colorless	Colorless	do	Russet	Russet	Chestnut.
Cornmeal	Warm buff	Ochraceous tawny to russet.	Chestnut to colorless.	Amber brown	Russet	Mummy brown	Liver brown	Mars brown.
Prune	do	Cinnamon brown to yellow ocher.	Chestnut	do	Colorless	do	do	Blackish brown.
Alfalfa	Buckthorn brown.	Buckthorn brown.	Sudan brown	Argus brown to buckthorn brown.	do	Prout's brown	Prout's brown	Russet.
Parsnip		Russet to cinnamon brown.	Chestnut	Chestnut	do	Mummy brown	Chestnut	Blackish brown.
Potato	Warm buff	Ochraceous tawny	Sayal brown	Sayal brown	Russet	Mummy brown	Blackish brown	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	11 days.	22 days.	38 days.	60 days.	11 days.	23 days.	38 days.	60 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot		Velvety	Velvety	Velvety		15×18	15×20	20×36
Malt	Velvety	do	do	do	5×7	20×35	20×40	20×65
Beet	do	do	do	do		15×25	18×30	20×35
Celery	do	do	do	do		18×20	20×25	20×32
Bean	Appressed	Appressed	Appressed	Appressed	1×2	10×18	10×20	20×30
Cornmeal	do	Velvety to appressed	Velvety to appressed	Velvety	1×2	18×30	20×35	20×55
Prune	Velvety	Velvety	Velvety	Velvety	5×7	20×30	20×40	20×60
Alfalfa	do	do	do	do	2×3	20×40	20×45	20×70
Parsnip		do	do	do		18×22	20×30	20×50
Potato	Velvety	do	do	do	2×3	10×10	10×12	12×15

TABLE III.—*Cultural data for Fomes texanus from Juniperus scopulorum (FP 21806), New Mexico*

[Subculture 2 from mycelium on alfalfa agar; initial culture from tissue. Series inoculated on Mar. 12, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	11 days.	22 days.	38 days.	70 days.	11 days.	22 days.	38 days.	70 days.
Carrot		Ochraceous tawny.	Amber brown	Amber brown to buckthorn brown.		Mummy brown	Liver brown	Blackish brown.
Malt	Warm buff	do	Chestnut to antimony yellow.	Chestnut to antimony yellow.		do	do	Do.
Beet	Light buff	Cinnamon brown to antimony yellow.	Chestnut to sodden.	Buckthorn brown to antimony yellow.		Blackish brown	Blackish brown	Blackish brown.
Celery	Buckthorn brown.	Antique brown to antimony yellow.	Argus brown	Argus brown		Auburn	do	Do.
Bean	Colorless	Colorless	Colorless	Colorless		Russet	Russet	Chestnut.
Corn meal	Light buff	Light buff to colorless.	Amber brown to colorless.	Amber brown	Russet	Mummy brown	Liver brown	Mars brown.
Prune	Warm buff	Cinnamon brown to yellow ocher.	Chestnut to yellow ocher.	Chestnut to amber brown.	Colorless	do	do	Blackish brown.
Alfalfa	Buckthorn brown.	Buckthorn brown to cinnamon brown.	Sudan brown	Argus brown	do	Prout's brown	Prout's brown	Mummy brown.
Parsnip		Russet to cinnamon brown.	Amber brown	Amber brown	do	Mummy brown	Chestnut	Blackish brown.
Potato	Light buff	Ochraceous tawny.	Sayal brown	Sayal brown	Russet	do	do	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	11 days.	22 days.	38 days.	70 days.	11 days.	22 days.	38 days.	70 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot		Velvety	Velvety	Velvety		15×25	20×38	20×72
Malt	Velvety	do	do	do	4×5	20×30	20×45	20×62
Beet	do	do	do	do	2× 1	15×15	18×20	20×45
Celery	do	do	do	do	10×20	20×25	20×32	20×40
Bean	Appressed	Appressed	Appressed	Appressed	3× 4	8× 8	15×25	20×40
Corn meal	do	Velvety to appressed	Velvety to appressed	Velvety	1× 2	20×20	20×40	20×60
Prune	Velvety	Velvety	Velvety	do	3× 5	20×25	20×38	20×60
Alfalfa	do	do	do	do	3× 4	20×30	20×40	20×65
Parsnip	do	do	do	do		15×20	20×35	20×55
Potato	Appressed velvety	Appressed velvety	Appressed velvety	Velvety	1× 2	8× 8	8×16	20×35

TABLE IV.—*Cultural data for Fomes texanus from Juniperus scopulorum (FP 21760), New Mexico*

[Subculture 3 from mycelium on corn meal agar; initial culture from tissue. Series inoculated on Mar. 12, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	11 days.	22 days.	38 days.	60 days.	11 days.	22 days.	38 days.	60 days.
Carrot		Cinnamon brown	Amber brown	Amber brown to buckthorn brown.		Mummy brown	Liver brown	Blackish brown.
Malt	Warm buff	Buckthorn brown.	Buckthorn brown to antimony yellow.	Buckthorn brown to antimony yellow.	Colorless	Chestnut	do	Do.
Beet		Prout's brown to cinnamon brown.	Chestnut	Chestnut		Blackish brown	Blackish brown	Do.
Celery		Warm buff	Sudan brown	Argus brown		Mummy brown	do	Do.
Bean	Colorless	Colorless	Colorless	Colorless	Colorless	Russet	Russet	Chestnut.
Corn meal	do	Russet to colorless.	Chestnut to colorless.	Amber brown to colorless.	Russet	Mummy brown	Liver brown	Mars brown.
Prune	Warm buff	Cinnamon brown to yellow ocher.	Chestnut to Sandford brown.	Chestnut to amber brown.	Colorless	Blackish brown	Blackish brown	Blackish brown.
Alfalfa	Buckthorn brown.	Buckthorn brown.	Sudan brown	Argus brown	do	Prout's brown	Prout's brown	Mummy brown.
Parsnip		Russet to cinnamon brown.	Chestnut	Amber brown to buckthorn brown.		Mummy brown	Chestnut	Blackish brown.
Potato		Ochraceous tawny.	Sayal brown	Sayal brown		Blackish brown	Blackish brown	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	11 days.	22 days.	38 days.	60 days.	11 days.	22 days.	38 days.	60 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot		Velvety	Velvety	Velvety		18×22	20×28	20×42
Malt	Velvety	do	do	do	1×2	20×25	20×40	20×62
Beet		do	do	do		5×10	10×12	10×15
Celery		do	do	do		15×20	20×25	20×35
Bean	Appressed	Appressed	Appressed	Appressed	1×2	10×20	15×25	20×40
Corn meal	do	Appressed to velvety	Appressed to velvety	Appressed to velvety	2×1	10×10	20×40	20×60
Prune	Velvety	Velvety	Velvety	Velvety	2×3	20×30	20×45	20×70
Alfalfa	do	do	do	do	4×6	20×32	20×42	20×62
Parsnip	do	do	do	do		12×20	20×40	20×60
Potato		do	do	do		8×15	10×18	10×22

TABLE V.—*Cultural data for Polyporus farlowii from Populus italica (FP 21402), New Mexico*

[Subculture 6 from mycelium on malt agar; initial culture from tissue]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	4 days.	16 days.	30 days.	63 days.	4 days.	16 days.	30 days.	63 days.
Carrot	Warm buff	Buckthorn brown.	Buckthorn brown.	Buckthorn brown.	Colorless	Colorless	Colorless	Colorless.
Malt	do	do	do	do	do	do	do	Do.
Beet	Colorless	do	do	do	do	do	do	Do.
Celery	White	do	do	do	do	do	Stained agar Sandford brown.	Stained agar Sandford brown.
Bean	do	White	White	White	do	do	Colorless	Colorless.
Corn meal		Buckthorn brown to yellow ocher.	Buckthorn brown.	Buckthorn brown.		do	do	Do.
Prune		Buckthorn brown.	do	do		do	do	Do.
Alfalfa		White	White	White		do	do	Do.
Parsnip		Buckthorn brown to antimony yellow.	Buckthorn brown.	Buckthorn brown.		do	do	Do.
Potato		Warm buff	Warm buff	Warm buff		do	do	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	4 days.	16 days.	30 days.	63 days.	4 days.	16 days.	30 days.	63 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Cottony	Woolly, dense	Woolly, dense	Woolly, dense	1×2	20×85	20×85	20×85
Malt		Woolly	Woolly	do		20×70	20×90	20×90
Beet	Appressed	Cottony	Woolly, dense	do	2×3	20×60	20×85	20×85
Celery	Cottony	Woolly	Woolly	do	4×5	20×80	20×90	20×90
Bean		Appressed to cobwebby	Silky	Silky to downy		20×50	29×75	20×80
Corn meal	Cottony	Velvety to felty	Woolly	Woolly, dense		20×70	20×80	20×80
Prune		do	Felty	Felty		20×50	20×85	20×90
Alfalfa		Cottony to silky dense	Woolly to silky	Felty to silky		20×70	20×90	20×90
Parsnip	Cottony	Dense cottony to woolly	Woolly, dense	Woolly, dense		20×75	20×90	20×90
Potato		Cottony	Cottony	Felty		20×20	20×75	20×75

TABLE VI.—*Cultural data for Polyporus farlowii from Acer negundo (FP 21591), New Mexico*

[Subculture 2 from mycelium on potato agar; initial culture from tissue. Series inoculated on Apr. 7, 1917]

Medium (agar).	Color of aerial mycelium.			Color of submerged mycelium.			Texture of aerial mycelium.			Size of area.		
	4 days.	36 days.	60 days.	4 days.	36 days.	60 days.	4 days.	36 days.	60 days.	4 days.	36 days.	60 days.
										Mm.	*Mm.*	*Mm.*
Carrot	Warm buff	Buckthorn brown.	Buckthorn brown.	Colorless.	Colorless.	Colorless.	Cottony to silky.	Woolly, dense.	Woolly, dense.	20×20	20×75	20×75
Malt	Antimony yellow.	do	do	do	do	do	Velvety	do	Felty	10×18	20×70	20×80
Beet	Yellow ocher	do	do	do	do	do	Cottony	Woolly to felty	do	10×25	20×60	20×80
Celery	Warm buff	do	do	do	do	do	do	Woolly, dense.	do	15×28	20×75	20×75
Bean	Colorless	Colorless	Colorless	do	do	do	Appressed, scant.	Appressed, scant.	Appressed, scant.	10×20	20×65	20×75
Corn meal	Warm buff	Buckthorn brown.	Buckthorn brown.	do	do	do	Cottony	Woolly, dense.	Woolly	15×25	20×90	20×90
Prune		do	do	do	do	do		Felty	Felty		20×65	20×65
Alfalfa	White	White	White	do	do	do	Cottony to silky.	Felty to silky.	Felty to silky.	15×25	20×75	20×75
Parsnip	Antimony yellow.	Buckthorn brown.	Buckthorn brown.	do	do	do	Cottony	Felty	Felty	15×20	20×85	20×85
Potato	Light buff	Warm buff	Warm buff	do	do	do	do	Woolly	do	15×15	20×70	20×70

DISCUSSION OF CULTURAL TABLES

Fomes texanus. The cultural characters for all of the four strains show remarkable uniformity for each of the corresponding agars in the series of ten. This fungus seems to be more susceptible to differences in the amount of sunlight it received than any species thus far investigated. When the intensity of the sunlight was diminished by the cheesecloth screens previously mentioned, this species on several of the agars immediately responded to the decrease in light by making a more vigorous growth and turning a lighter shade of brown.

Polyporus farlowii. This fungus is also very uniform in growth on each of the 10 agars as the tables show. When grown in the dark, the strain from *Acer negundo* developed a submerged mycelium on prune and corn-meal agars which was Mars brown in place of colorless. Whether this change in colors of the mycelium was due to the darkness is doubtful since the other strain from *Populus italica* when grown in darkness still retained its colorless mycelium the same as when grown in the light.

Series were grown of nine strains of *Trametes pini* obtained from material collected in four States and growing on seven hosts—viz, *Pinus echinata*, *P. flexilis*, *P. ponderosa*, *Picea engelmannii*, *Pseudotsuga taxifolia*, *Abies arizonica*, and *A. lasiocarpa*. This series represented subcultures ranging from 1 to 7. While there was some slight variation, especially in the colors of the mycelium of the various strains, the differences were not so marked as to constitute real specific characters. There was practically no difference between the cultural characters obtained from the different subcultures. This would indicate that the general fundamental characters of the fungus are not materially changed through successive subcultures, at least in this instance to the seventh subculture.

As will be noted from the temperature record, there was rather a wide variation in temperature during the time the various series of cultures were growing. Nevertheless the general cultural characters as shown in tables are practically identical. This identity of characters for a given organism on a given culture medium is still more marked when a series of from three to six tubes of the same agar for the same strain is made at the same time and then compared as the growth progresses.

CULTURAL CHARACTERS FOR DIFFERENT STRAINS OF SAME FUNGUS

The sources of the initial cultures from the nine strains of *Trametes pini* were five from sporophore tissue and four from infected wood, representing seven hosts, while the cultures from *Fomes texanus* all were from tissue but from two hosts. The *Polyporus farlowii* cultures were both from tissue but from different hosts. A careful comparison of the cultural characters of the various strains of each of these fungi shows no appreciable differences between cultures of a given fungus whether obtained from infected wood or from sporophores; neither do the hosts

of the fungus seem to make any marked changes in the fundamental cultural characters, as is clearly shown in the various tables when strains from different hosts are compared. There may be minor differences due to the host from which the strain came, but nothing more.

The comparison of the cultural characters of many species of parasitic fungi has long been recognized as a reliable index to the identity of the fungus under investigation, and there is no reason why the cultural characters of wood-rotting fungi which are just as uniform and dependable should not be used for identification purposes.

The writers have purposely avoided going into a discussion of the results of the use of synthetic agars and of other special media, as these will be taken up in a later article. They have presented here the results obtained from agars easily made and apparently of a uniform enough composition for similar cultural characters to appear on different batches of the same agar even when the acidity, alkalinity, and water content vary considerably. Just how great a variation in these factors must occur to produce a decided change in the cultural characters is a problem for future investigation.

EXAMPLES OF THE DIAGNOSTIC VALUE OF CULTURAL CHARACTERS IN SPECIES DETERMINATION

In comparing the cultural characters of closely related but really distinct species marked and constant differences in the character of the mycelium will be found on certain corresponding agars in the series of cultures representing the two species, while if the two fungi are really the same species no constant differences of specific rank will be found. The following fungi will illustrate the diagnostic value of the cultural characters in determining the real position of the species.

TABLE VII.—*Cultural data for Fomes rimosus from Acacia roemeriana (FP 21681), Texas*

[Subculture 3 from mycelium on malt agar; initial culture from tissue. Series inoculated on May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Clay color to light buff.	Clay color to warm buff.	Clay color to warm buff.	Clay color	Colorless	Colorless	Colorless	Colorless.
Malt	Clay color to warm buff.	Amber brown to buckthorn brown.	Amber brown to buckthorn brown.	Amber brown	do	Amber brown	Amber brown to colorless.	Amber brown to colorless.
Beet	Sodden	Light buff to sodden	Warm buff to mainly sodden.	Warm buff to mainly sodden.	do	Colorless 11–15 mm. beyond aerial mycelium.	Colorless 20–30 mm. beyond aerial mycelium.	Colorless 40–45 mm. beyond aerial mycelium.
Celery	Warm buff to colorless.	Warm buff to colorless.	Warm buff to mainly colorless.	Warm buff to mainly colorless.	do	Colorless 20–30 mm. beyond aerial mycelium.	Colorless 40–45 mm. beyond aerial mycelium.	Colorless 45–50 mm. beyond aerial mycelium.
Bean	Sodden	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	Colorless 10–12 mm. beyond aerial mycelium.	Colorless 20–30 mm. beyond aerial mycelium.	Colorless 25–32 mm. beyond aerial mycelium.
Corn meal	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	do	Colorless	Colorless	Colorless.
Prune	do	Buckthorn brown to warm buff.	Buckthorn brown.	Buckthorn brown.	do	Tawny to colorless.	Tawny to colorless.	Tawny to colorless.
Alfalfa	Light buff	Maize yellow to colorless.	Maize yellow to colorless.	Warm buff to mainly colorless.	do	Colorless	Colorless	Colorless.
Parsnip	Buckthorn brown to warm buff.	Buckthorn brown to light buff.	Buckthorn brown.	Buckthorn brown.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Subfelty to silky	Felty	Felty	Felty	20×32	20×58	20×78	20×78
Malt	Subfelty	Felty to downy	Felty to downy	Felty	20×26	20×60	20×75	20×75
Beet	Appressed	Downy to mainly appressed	Downy to mainly appressed	Downy to mainly appressed	15×15	20×40	20×55	20×57
Celery	Subfelty to appressed	Felty to mainly appressed	Felty to mainly appressed	Felty to mainly appressed	10×15	20×45	20×70	20×70
Bean	Appressed	Downy to mainly appressed	Downy to mainly appressed	Downy to mainly appressed	10×15	20×35	20×50	20×55
Corn meal	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to appressed	20×25	20×46	20×60	20×75
Prune	do	Subfelty to downy	Felty to downy	Felty to downy	20×38	20×60	20×70	20×75
Alfalfa	Cottony-silky	Silky-felty to appressed	Silky-felty to appressed	Silky-felty to appressed	10×15	20×32	20×45	20×75
Parsnip	Felty	Felty to appressed	Felty to appressed	Felty to appressed	20×30	20×55	20×75	20×82
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	15×18	20×25	20×26	20×27

TABLE VIII.—*Cultural data for Fomes rimosus from Acacia roemeriana (FP 21817), Texas*

[Subculture 1 from mycelium on prune agar; initial culture from infected wood. Series inoculated on May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to light buff.	Clay color to warm buff.	Clay color to warm buff.	Clay color to warm buff.	Colorless	Colorless	Colorless	Colorless
Malt	Clay color to warm buff.	Buckthorn brown to white.	Amber brown to buckthorn brown.	Amber brown to buckthorn brown.	do	Amber brown	Amber brown to colorless.	Amber brown to colorless.
Beet	Sodden	Warm buff to sodden.	Buckthorn brown to mainly sodden.	Buckthorn brown to mainly sodden.	do	Colorless 10–12 mm. beyond aerial mycelium.	Colorless 15–20 mm. beyond aerial mycelium.	Colorless 10–15 mm. beyond aerial mycelium.
Celery	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless 20–30 mm. beyond aerial mycelium.	Colorless 40 mm. beyond aerial mycelium.	Colorless 30 mm. beyond aerial mycelium.
Bean	Light buff	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 30–35 mm. beyond aerial mycelium.	Colorless 25–30 mm. beyond aerial mycelium.
Corn meal	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.
Prune	Clay color to colorless.	Buckthorn brown to warm buff.	Buckthorn brown to white.	Buckthorn brown to light buff.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Alfalfa	Light buff	Maize yellow to colorless.	Maize yellow to colorless.	Warm buff to mainly colorless.	do	Colorless 10–15 mm. beyond aerial mycelium.	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 25–30 mm. beyond aerial mycelium.
Parsnip	Buckthorn brown to warm buff.	Buckthorn brown to light buff.	Buckthorn brown.	Buckthorn brown.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	Light buff to colorless.	Light buff to colorless.	Light buff to colorless.	Light buff to colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Subfelty to silky	Felty	Felty	Felty	20×25	20×58	20×75	20×75
Malt	Subfelty to downy	Felty to downy	Felty to downy	do	20×25	20×25	20×65	25×75
Beet	Appressed	Downy to appressed	Downy to mainly appressed	Downy to mainly appressed	12×10	20×22	20×28	20×30
Celery	Subfelty	Felty to mainly appressed	Felty to mainly appressed	Felty to mainly appressed	10×12	20×50	20×70	20×70
Bean	Downy	Downy to mainly appressed	Downy to mainly appressed	Downy to mainly appressed	15×18	20×45	20×50	20×55
Corn meal	Downy-felty to mainly appressed.	Downy-felty to mainly appressed.	Downy-felty to mainly downy appressed.	Downy-felty to mainly downy appressed.	18×25	20×42	20×50	20×56
Prune	Downy-felty to downy	Subfelty to downy	Felty to downy	Felty to downy		20×20	20×55	20×72
Alfalfa	Subfelty to silky	Silky-felty to appressed	Silky-felty to appressed	Silky-felty to appressed		12×15	20×60	20×60
Parsnip	Velvety-felty	Felty to appressed	Felty to downy	Felty		15×22	20×62	20×72
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to felty		15×20	20×32	20×33

TABLE IX.—*Cultural data for Fomes rimosus from Siderocarpos flexicaulis (FP 21741), Texas*

[Subculture 2 from mycelium on malt agar; initial culture from tissue. Series inoculated on May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to light buff.	Clay color to warm buff.	Clay color	Amber brown	Colorless	Colorless	Colorless	Colorless.
Malt	Clay color to warm buff.	Amber brown to white.	Amber brown to buckthorn brown.	do	Old mycelium buckthorn brown.	Amber brown to colorless.	Amber brown to colorless.	Amber brown to colorless.
Beet	Sodden	Light buff to sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	Colorless	Colorless 20–22 mm. beyond aerial mycelium.	Colorless 25–30 mm. beyond aerial mycelium.	Colorless 20–30 mm. beyond aerial mycelium.
Celery	Clay color to light buff.	Clay color to colorless.	Buckthorn brown to colorless.	Buckthorn brown to colorless.	do	do	Colorless 15–20 mm. beyond aerial mycelium.	Colorless 20–25 mm. beyond aerial mycelium.
Bean	Light buff	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	do	do	Colorless 15–20 mm. beyond aerial mycelium.
Corn meal	Sayal brown to colorless.	Buckthorn brown to colorless.	Buckthorn brown to colorless.	Buckthorn brown to colorless.	do	Clay color	Clay color to colorless.	Clay color to colorless.
Prune	Clay color to colorless.	do	do	Amber brown	do	Cinnamon brown to colorless.	Cinnamon brown to colorless.	Cinnamon brown to colorless.
Alfalfa	Clay color to light buff.	Buckthorn brown to sodden.	Buckthorn brown to sodden.	Buckthorn brown tosodden.	do	Colorless 10–12 mm. beyond aemycelium.	Colorless 6–8 mm. beyond aerial mycelium.	Colorless 20 mm. beyond aerial mycelium.
Parsnip	Warm buff to colorless.	Argus brown to light buff.	Argus brown	Argus brown	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	do	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Subfelty to silky	Felty	Felty	Felty	20×32	20×62	20×72	20×72
Malt	Downy-felty to appressed	Felty to downy	Felty to downy	do	20×25	20×60	20×75	20×75
Beet	Appressed	Downy to mainly appressed	Downy mainly appressed	Downy mainly appressed	15×15	20×32	20×55	20×60
Celery	Subfelty	Felty to appressed	Felty to appressed	Felty to appressed	10×16	20×40	20×55	20×60
Bean	Downy	Downy to appressed	Downy to mainly appressed	Downy to mainly appressed	15×15	20×40	20×55	20×60
Corn meal	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to downy	18×20	20×45	20×60	20×65
Prune	do	Subfelty to appressed	Felty to appressed	Felty to downy	20×25	20×50	20×70	20×75
Alfalfa	Subfelty to silky	Felty to appressed	do	Felty to appressed	10×12	20×30	20×50	20×65
Parsnip	Downy to appressed	Subfelty to downy	Felty to downy	Felty	10×12	20×45	20×65	20×75
Potato	do	Downy to appressed	Downy to appressed	Downy to appressed	20×15	20×25	20×26	20×26

TABLE X.—*Cultural data for Fomes rimosus from Prosopis juliflora (FP 21687), Texas*

[Subculture 2 from mycelium on beet agar; initial culture from tissue. Series inoculated May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to light buff.	Clay color to warm buff.	Clay color to warm buff.	Amber brown to antimony yellow.	Colorless.	Colorless.	Colorless.	Colorless.
Malt	Clay color to warm buff.	Amber brown to white.	Amber brown to buckthorn brown.	Amber brown to light buff.	Buckthorn brown.	Amber brown to colorless.	Amber brown to colorless.	Amber brown to colorless.
Beet	Sodden.	Light buff to sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	Colorless.	Colorless 10–15 mm. beyond aerial mycelium.	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 20–25 mm. beyond aerial mycelium.
Celery	Light buff to colorless.	Light buff to colorless.	Light buff to colorless.	Light buff to colorless.	do.	Colorless 15–20 mm. beyond aerial mycelium.	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 25–30 mm. beyond aerial mycelium.
Bean	Light buff.	Light buff to sodden.	Light buff to sodden.	Light buff to sodden.	do.	Colorless 10–15 mm. beyond aerial mycelium.	Colorless 15–20 mm. beyond aerial mycelium.	Colorless 10–15 mm. beyond aerial mycelium.
Corn meal	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	do.	Clay color to mainly colorless.	Clay color to mainly colorless.	Clay color to mainly colorless.
Prune	Light buff to colorless.	Light buff to white.	Clay color to white.	Clay color to white.	do.	Ochre to colorless.	Ochre to colorless.	Ochre to colorless.
Alfalfa	Clay color to light buff.	Buckthorn brown to light buff.	Buckthorn brown to colorless.	Buckthorn brown to colorless.	do.	Colorless 10–12 mm. beyond aerial mycelium.	Colorless 6–8 mm. beyond aerial mycelium.	Colorless 6–8 mm. beyond aerial mycelium.
Parsnip	Colorless.	Clay color to mainly light buff.	Clay color to mainly light buff.	Buckthorn brown to light buff.	do.	Colorless.	Colorless.	Tawny to mainly colorless.
Potato	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do.	do.	do.	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Silky-felty to silky	Felty	Felty	Felty	20×35	20×65	20×70	20×70
Malt	Downy-felty to appressed	Felty to downy	Felty to downy	do	20×26	20×50	20×60	20×65
Beet	Appressed	Downy to mainly appressed.	Downy to mainly appressed.	Downy to mainly appressed.	8×10	20×32	20×40	20×45
Celery	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	20×30	20×50	20×70	20×70
Bean	Downy	do	do	do	15×20	20×30	20×35	20×42
Corn meal	Downy to appressed	do	do	do	18×20	20×45	20×55	20×57
Prune	do	Subfelty to downy	Subfelty to downy	Subfelty to downy	20×25	20×60	20×70	20×72
Alfalfa	Subfelty to silky	Felty to appressed	Felty to appressed	Felty to appressed	10×18	20×42	20×55	20×56
Parsnip	Appressed	Subfelty to downy	Felty to downy	Felty to downy	10×15	20×50	20×70	20×75
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	20×20	20×30	20 35	20×36

TABLE XI.—*Cultural data for Fomes robiniae from Robinia neomexicana (FP 21779), New Mexico*

[Subculture 2 from mycelium on parsnip agar; initial culture from infected wood. Series inoculated May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to light buff.	Clay color to warm buff.	Clay color to warm buff.	Clay color to antimony yellow.	Colorless	Colorless	Colorless	Colorless.
Malt	Clay color to light buff.	Amber brown to white.	Amber brown to buckthorn brown.	Amber brown	do	Amber brown	Amber brown to colorless.	Amber brown to colorless.
Beet	Light buff to sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 35–40 mm. beyond aerial mycelium.	Colorless 35–40 mm beyond aerial mucelium.
Celery	Clay color to colorless.	Buckthorn brown to colorless.	Buckthorn brown to mainly colorless.	Buckthorn brown to mainly colorless.	do	do	do	Do.
Bean	Colorless	Light buff to sodden.	Light buff ro sodden.	Light buff to sodden.	do	do	do	Colorless 20–25 mm. beyond aerial mycelium.
Corn meal	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	do	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.
Prune	do	Buckthorn brown to colorless.	Buckthorn brown to colorless.	Amber brown to colorless.	do	Cinnamon brown to colorless.	Cinnamon brown to colorless.	Cinnamon brown to colorless.
Alfalfa	Clay color to colorless.	do	Buckthorn brown to light buff.	Buckthorn brown to light buff.	do	Colorless 5–8 mm. beyond aerial mycelium.	Colorless 6–8 mm. beyond aerial mycelium.	Colorless 6–8 mm. beyond aerial mycelium.
Parsnip	Buckthorn brown to warm.	Amber brown to light buff.	Sandford brown to warm buff.	Sandford brown to warm buff.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					mm.	mm.	mm.	mm.
Carrot	Felty	Felty	Felty	Felty	20×40	20×70	20×75	20×75
Malt	Subfelty	Felty to downy	do	do	20×35	20×65	20×80	20×80
Beet	Downy-silky to appressed.	Downy to mainly appressed.	Downy to mainly appressed.	Downy to mainly appressed.	15×15	20×50	20×60	20×65
Celery	Downy to appressed	Downy to appressed	Subfelty to appressed	Subfelty to appressed	20×28	20×55	20×75	20×75
Bean	Appressed	Appressed	Downy to mainly appressed.	Downy to mainly appressed.	20×25	20×35	20×50	20×52
Corn meal	Downy-felty to appressed.	Downy-felty to appressed.	Downy-felty to appressed.	Downy-felty to appressed.	20×32	20×52	20×62	20×63
Prune	do	Subfelty to downy	Subfelty to downy	Subfelty to downy	20×35	20×65	20×75	20×75
Alfalfa	Subfelty to silky	Felty to appressed	Felty to appressed	Felty to appressed	15×18	20×42	20×58	20×58
Parsnip	Velvety-felty	Felty to downy	Felty to downy	Felty to downy	20×28	20×60	20×82	20×82
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	18×20	20×30	20×35	20×36

TABLE XII.—*Cultural data for Fomes rimosus from Juglans rupestris(?) (FP 21611), Arizona*

[Subculture 3 from mycelium on carrot agar; initial culture from tissue. Series inoculated May 29, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to warm buff.	Clay color to warm buff.	Clay color to warm buff.	Clay color to warm buff.	Colorless	Colorless	Colorless	Colorless.
Malt	Clay color to warm buff.	Buckthorn brown to antimony yellow to warm buff.	Buckthorn brown.	Buckthorn brown.	do	Amber brown	Amber brown	Amber brown.
Beet	Warm buff to sodden.	Warm buff to sodden.	Buckthorn brown to sodden.	Buckthorn brown to sodden.	do	Colorless 10 – 15 mm. beyond aerial mycelium.	Colorless 15 – 30 mm. beyond aerial mycelium.	Colorless 10–12 mm. beyond aerial mycelium.
Celery	Warm buff to colorless.	Warm buff to colorless.	Warm buff to mainly colorless.	Warm buff to mainly colorless.	do	Colorless 20 – 30 mm. beyond aerial mycelium.	Colorless 35 – 40 mm. beyond aerial mycelium.	Colorless 40 – 45 mm. beyond aerial mycelium.
Bean	Light buff	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	Colorless 10 – 15 mm. beyond aerial mycelium.	Colorless 20 – 30 mm. beyond aerial mycelium.	Colorless 20 – 25 mm. beyond aerial mycelium.
Corn meal	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.
Prune	Light buff to colorless.	do	Clay color to white	Clay color to white	do	Cinnamon brown to colorless.	Cinnamon brown to colorless.	Cinnamon brown colorless.
Alfalfa	Light buff	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless 10 – 15 mm. beyond aerial mycelium.	Colorless 20 – 25 mm. beyond aerial mycelium.	Colorless 20 – 25 mm. beyond aerial mycelium.
Parsnip	Antimony yellow to colorless.	Sandford brown to to warm buff.	Sandford brown to to warm buff.	Sandford brown to to warm buff.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	Light buff to colorless.	Light buff to colorless.	Light buff to mainly colorless.	Light buff to mainly colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Felty to silky	Felty	Felty	Felty	20×35	20×72	20×80	20×80
Malt	Felty to appressed downy	Felty to downy	Felty to downy	Dense felty to downy	20×22	20×60	20×85	20×85
Beet	Silky	Downy to mainly appressed.	Downy to mainly appressed.	Downy to mainly appressed.	5×10	10×30	20×30	20×31
Celery	Downy to appressed	do	do	do	10×15	20×35	20×50	20×75
Bean	Downy	do	do	do	8×10	20×26	20×40	20×42
Corn meal	Downy to mainly appressed.	Downy to mainly appressed.	do	do	15×18	20×35	20×60	20×70
Prune	Downy to appressed	Downy to appressed	Downy-appressed	Downy-appressed	15×18	20×48	20×70	20×72
Alfalfa	Cottony	Silky-felty to appressed	Silky-felty to appressed	Silky-felty to mainly appressed.	10×15	20×30	20×40	20×60
Parsnip	Felty	Felty to appressed	Felty to appressed	Felty to appressed	20×25	20×55	20×85	20×85
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	15×12	20×25	20×40	20×45

TABLE XIII.—*Cultural data for Folyporus dryophilus from Quercus gambelii (FP 21598), New Mexico*

[Subculture 2 from mycelium on beet agar; initial culture from infected wood. Series inoculated on June 1, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Buckthorn brown to light buff.	Buckthorn brown.	Buckthorn brown.	Buckthorn brown.	Colorless.	Colorless.	Cinnamon brown to colorless.	Cinnamon brown to colorless.
Malt	do.	do.	do.	do.	do.	Tawny to colorless.	Tawny.	Tawny to colorless.
Beet	White.	Light buff to white	Light buff to white	Buckthorn brown to white.	do.	Colorless.	Colorless.	Colorless.
Celery	Light buff to white	do.	Warm buff to white.	Warm buff to mainly white.	do.	do.	do.	Do.
Bean	White.	do.	Light buff to white	Light buff to white	do.	do.	do.	Do.
Corn meal	Clay color to mainly light buff.	Clay color to mainly light buff.	Clay color to mainly light buff.	Clay color to mainly light buff.	do.	do.	do.	Do.
Prune	Warm buff to light buff.	Clay color to light buff.	Clay color to light buff.	Buckthorn brown.	do.	Tawny to mainly colorless.	Tawny to mainly colorless.	Tawny to mainly colorless.
Alfalfa	White.	Light buff to white	Light buff to white	Light buff to white	do.	Colorless.	Colorless.	Colorless.
Parsnip	Buckthorn brown to light buff.	Buckthorn brown.	Buckthorn brown.	Buckthorn brown.	Tawny.	Tawny.	Tawny.	Tawny.
Potato	White.	White.	White.	White.	Colorless.	Colorless.	Colorless.	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Dense woolly	Dense woolly	Dense woolly	Dense woolly	20×52	20×80	20×80	20×80
Malt	do.	do.	do.	do.	20×50	20×75	20×75	20×75
Beet	Cottony	Cottony	Cottony-woolly	Cottony-woolly	18×25	20×45	20×55	20×55
Celery	do.	Cottony-woolly	do.	do.	20×32	20×52	20×56	20×57
Bean	do.	Cottony to downy	Cottony-felty	Cottony-felty	20×28	20×50	20×65	20×70
Corn meal	do.	Cottony-woolly	Cottony-woolly	Woolly	20×46	20×75	20×75	20×80
Prune	Woolly	Woolly	Woolly	do.	20×40	20×80	20×80	20×80
Alfalfa	Woolly to silky	Woolly-silky	Woolly-silky	Woolly-silky	20×28	20×50	20×60	20×61
Parsnip	Woolly, dense	Woolly, dense	Woolly, dense	Woolly, dense	20×50	20×80	20×80	20×80
Potato	Cottony to silky	Cottony to downy	Cottony to downy	Cottony to downy	20×25	20×27	20×28	20×29

TABLE XIV.—*Cultural data for Polyporus texanus from Prosopis juliflora (FP 21201), Texas*

[Subculture 1 from mycelium on malt agar; initial culture from tissue. Series inoculated on May 29, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Clay color to light buff.	Clay color to light buff.	Clay color to light buff.	Clay color to light buff.	Colorless	Colorless	Colorless	Colorless.
Malt	do	Buckthorn brown to light buff.	Buckthorn brown to light buff.	Buckthorn brown to light buff.	do	do	do	Do.
Beet	White	White	Light buff to white	Light buff to sodden.	do	do	do	Do.
Celery	Light buff to white	Clay color to white.	Clay color to white.	Clay color to white.	do	do	do	Do.
Bean	Light buff	Light buff	Light buff	Light buff	do	do	do	Do.
Corn meal	Ochraceous buff to light buff.	Clay color to light buff.	Clay color to light buff.	Clay color to white.	do	do	do	Do.
Prune	Clay color to light buff.	Clay color to white.	Clay color to white.	Clay color	do	do	do	Do.
Alfalfa	White	Warm buff to white.	Warm buff to white.	Warm buff to white.	do	do	do	Do.
Parsnip	Clay color to light buff.	Clay color to white.	Clay color to white.	Buckthorn brown to clay color.	do	do	do	Do.
Potato	Warm buff to white.	Clay color to light buff.	Clay color to light buff.	Clay color to mainly light buff.	do	do	do	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Cottony, coarse plumose	Cottony to felty	Cottony to felty	Cottony to felty	20×30	20×50	20×60	20×67
Malt	do	Cottony to cottony-felty	Cottony-felty	Felty strong plumose tufts.	20×28	20×50	20×65	20×75
Beet	Cottony	Cottony	do	Felty to appressed	8×12	15×18	20×22	20×25
Celery	Cottony, plumose	Cottony-felty	do	Felty to downy, plumose tufts.	20×20	20×37	20×45	20×50
Bean	Cottony	Cottony, plumose	Cottony, plumose	Cottony, plumose	[a] 8×8	[a] 8×8	[a] 8×8	[b] 8×8
Corn meal	Cottony, plumose	Cottony	Cottony to felty	Felty to downy, plumose tufts.	15×20	20×48	20×65	20×70
Prune	do	Cottony-felty to downy	Cottony-felty to downy	Felty to downy strong plumose tufts.	20×30	20×50	20×65	20×68
Alfalfa	do	Cottony	Cottony to felty	Felty to downy, plumose tufts.	15×20	20×35	20×45	20×47
Parsnip	do	Cottony to felty-cottony	do	Felty, plumose tufts	20×28	20×54	20×65	20×75
Potato	do	do	do	Felty to downy	20×25	20×40	20×60	20×70

[a] Mainly on inoculum.

[b] Practically no growth.

Tables VII to XII show the cultural characters on the 10 agars for Fomes *rimosus* (two strains) from *Acacia roemeriana* (catclaw), *F. rimosus* from *Siderocarpos flexicaulis* (Texas ebony), *F. rimosus* from *Prosopis juliflora* (mesquite), *F. robiniae* from *Robinia neomexicana* and a species of Fomes from *Juglans rupestris* (?). A study of these six tables fails to show any marked and constant differences in cultural characters for any of the 10 agars for the six strains given, although four of the six strains represent *Fomes rimosus*, one *F. robiniae* and one a Fomes on *Juglans rupestris* (?) which the writers have been referring to *F. everhartii*. Some of the cultures on carrot, malt, and parsnip show slight color differences, but none great enough to be of any specific value. On parsnip and malt, the submerged mycelium for all of these strains is colored, while carrot has colored to colorless mycelium. The dominant characteristics of *Fomes rimosus* is seen on beet, celery, bean, and alfalfa agars. On each of these agars there is but little really aerial mycelium which is downy to mainly appressed, while the submerged mycelium extends far beyond the aerial mycelium, giving a peculiar and very characteristic glassy appearance to the surface of the agar. This peculiarity of growth of the submerged mycelium on these four agars is seen in all of the six strains and indicates that they are one and the same species—viz, that *Fomes robiniae* is only a form of *F. rimosus*, as many scientists have always believed, and that the specimen of Fomes sp. supposed to have been collected on *Juglans rupestris* also belongs to this species. These six examples show how valuable the cultural characters can become in determining the specific position of closely related or identical species. Only one viable sporophore of the Fomes supposed to have been collected on *J. rupestris* was available for culture work, and since this specimen may have been wrongly labeled as to host, the writers do not wish to place the Fomes so common on *J. rupestris* throughout the southwestern United States as belonging positively to *Fomes rimosus* until further cultures with specimens absolutely known to have grown on this host have been made.

The cultural characters of *Fomes everhartii*, *F. arctostaphyli* (10), *F. igniarius* from *Populus tremuloides*, and a species of Fomes from Alnus sp., sent to the writers as *F. igniarius*, when compared showed that the Fomes from Alnus is more closely related to *F. arstostaphyli* than to *F. igniarius* from *P. tremuloides*, but that it is apparently neither of these two species; nor did it have any of the cultural characters of *F. everhartii*. In this instance the sporophore from *Alnus* sp. was very similar in all its characters to the sporophore of *F. igniarius* from *P. tremuloides*, yet the cultural characters instantly showed the two were not the same species.

Polyporus farlowii, *P. dryophilus*, and *P. texanus* are three closely related species which differ but little in their general sporophore characters. Tables V, VI, XIII, and XIV show the cultural characters of each

of these three fungi. The cultural differences between *P. texanus* and *P. dryophilus* are much more marked than those between *P. farlowii* and *P. dryophilus*. A study of the tables giving the characters of these three fungi at once shows marked and constant differences between each of the three species. It will be seen that general color resemblances are not as distinctive in differentiating specific characters as are certain other factors. Many of these species have a buckthorn brown color on several of the agars, which indicates their general relationship while specific characters must be sought in the differences of growth on certain agars.

The difference in the fruiting of related species on the various agars is also of much value in differentiating species. For instance, *P. farlowii* fruits vigorously on several of the ten culture media producing perfect and typical pores, *P. dryophilus* very rarely fruits on the culture media here given unless the inoculum is fresh sporophore tissue, while *P. texanus* has so far fruited on only two of them.

IDENTIFICATION OF UNKNOWN ROTS BY CULTURAL CHARACTERS

The cultural character method here given can be used to determine what fungus produces a given rot. In a large majority of cases rots, both heart and saprophytic, will be found without any sporophores being present to indicate what fungus produced the rot. If careful inoculations are made from such infected wood, it is a comparatively easy matter, as a rule, to obtain pure cultures of the causative organisms and later grow them again on the 10 media given, thus determining their cultural characters and from them the fungi producing the rots.

The number of species of fungi producing heartrots in living trees is not very great. A few of them produce rots which can usually be identified by the character of the rot alone, such as *Trametes pini*, but the great majority of them can not be certainly determined by the rot. For instance, the rot produced in conifers by *Polyporus schweinitzii*, *P. sulphureus*, and *Fomes laricis* are so similar that no one can be certain by examining the rot alone which of these three fungi was the cause of the rot in question.

The rots of structural timbers are more numerous than the true heartrots and the causative organism producing each rot is more difficult to determine from the rot alone, since the majority of the structural-timber rots are very similar in general appearance. Most of these rots are of the carbonizing type, where a brownish, brittle rot is produced which on dessication breaks up into little cubical blocks of varying sizes. Pure cultures from these structural timber rots will differentiate them as to their causative organisms at once. The value of such means of determining the causative organism from the rot in the absence of any sporophore is very evident to anyone who has had to deal with such rots.

EXAMPLES OF IDENTIFICATION OF UNKNOWN ROTS BY CULTURAL CHARACTERS

The following examples illustrate how the cultural characters here described may be used to determine the causative organism of unknown rots:

(*a*) There are two common heartrots found in conifers in the western United States. One is the redrot caused by *Trametes pini* and the other is what the senior author has previously called western redrot (*9*). In their early stages of growth these two rots resemble each other very much. Pure cultures from each of them differentiate the two immediately, since the pure cultures of the fungus which causes western redrot, called previously *Polyporus ellisianus*, produces entirely white cultures on all of the 10 media, while the cultures of *Trametes pini* are varying shades of brown.

(*b*) On a recent field trip in eastern Texas, when the senior writer was studying the rots of bridge timbers and railroad ties, he often found a certain rot in driven bridge piling made of creosoted longleaf pine (*Pinus palustris*). At that time in the year there were no fruiting bodies present on the rotting piling and the question at once arose as to what fungus was the cause of this serious rot. From the peculiar odor of the freshly opened wood as well as the character of the rot it was believed that it was caused by *Lentinus lepideus*, but nothing could be determined definitely from an examination of the rot alone. Specimens of the rotted piling were forwarded to the laboratory and cultures made from them. Pure cultures were obtained showing all of the characters of *L. lepideus* when grown on the 10 cultural media. Later many of these cultures developed the typical sporophores of *L. lepideus*. Cultures of this fungus can usually be recognized by the presence of abortive sporophores which develop on the surface of the agar and also by the presence of large, thick-walled, obovate to subglobose spores in the submerged mycelium.

(*c*) While studying the rots of cypress (*Taxodium distichum*) ties in wet locations in eastern Texas two unknown rots were found in this wood. Pieces of the rotting ties were sent to the laboratory and pure cultures of two different fungi were obtained. One set of these cultures showed all the cultural characters of *Lentinus lepideus*, while the other series produced a sporophore of an unknown species of Poria.

(*d*) A rot in the heartwood of *Quercus gambelii*, collected in New Mexico, was determined in the field as caused by *Polyporus dryophilus*. Cultures of the diseased wood gave all of the cultural characters of *Fomes everhartii* and none of those of *P. dryophilus*.

(*e*) A specimen of rot in *Pinus ponderosa*, which was supposed to be caused by *Polyporus sulphureus*, was received from Oregon. Cultures from the wood showed that the rot was unquestionably caused by

Lentinus lepideus. Since the rot was sent in as a specimen of heartrot found in the western yellow pine in Oregon, the writers are wondering whether *L. lepideus* produces a real heartrot of living pine in that State or whether this rot came from a dead area on a living or from a dead tree which had been attacked by this saprophytic fungus.

Many other instances could be cited of the determination of the causative organism of a rot by use of the cultural methods here outlined. Usually it is not even necessary that the sporophore stage should be developed, since the vegative cultural characters on the 10 special media will usually determine the identity of the fungus. The practical importance of such a method of determination is of great value and is easily recognized by anyone who has worked for any length of time with organisms of this character. In fact, one of the worst stumbling blocks to a successful study of the various rots of wood, both saprophytic and heart rots, has been the lack of methods by which the organisms producing these rots could be grown and identified in pure cultures on artificial media.

SPOROPHORE PRODUCTION

The fact has long been known that the production of sporophores in nature in many of the Hymenomycetes was more or less dependent upon light. This fact has also been demonstrated for a few species mainly by gross cultures on dung decoctions, pieces of wood, bread, etc. Buller (*4*) has shown among other things how the light influences sporophore production for a few species, mainly Agaricaceae, but none of his experiments were made with pure cultures on artificial media. It has been taken for granted that light was essential to the formation of sporophores of the wood-rotting fungi, including the Polyporaceae, but such had never been proved with pure cultures under control conditions on artificial media with a sufficiently large number of species to determine the actual influence of light as well as other factors on sporophore production.

The studies here made indicate that there are many Polyporaceae which fruit in diffused light of varying degrees of intensity and others apparently require the direct rays of the sun to produce perfect spore-bearing sporophores, while some can form sporophores in absolute darkness. Since this is only a preliminary report, no attempt is made to determine a large number of factors which should be ascertained in a complete study of this phase of fungus life. For instance, there must be a minimum, optimum, and maximum condition as to light, heat, and moisture under which a given fungus will produce sporophores.

It will be seen by consulting Table XVI that only three species, *Polyporus farlowii*, *Trametes serialis*, and *P. cinnabarinus*, were able to develop sporophores in absolute darkness. Only the first two of these fungi produced both sporophores and spores. *P. cinnabarinus* produced fairly typical pores, but no spores were found.

METHODS OF EXPERIMENTATION

In the earlier sporophore study the tubes containing the cultures were kept both horizontally and vertically. If the species under investigation produced sporophores at all, they were able to produce them in either position. However, the practice was soon abandoned of placing the tubes in a vertical position, since it was found very difficult to obtain uniformity in the proper lighting of the cultures and to get spore prints from the sporophores produced in such a position.

The general method followed in the sporophore studies was to take a series of tubes on different agars and place them in the same general position that was described in the study of the cultural characters. It was found very important early in the study that the slant side of the tube should be kept uppermost and that the relative position of the tube in reference to gravity and sunlight should be the same throughout the experiment. The tubes were so placed that the cotton plugs were away from the sunlight and the bottom of the tubes faced the light. As soon as there was any indication of a hymenium forming, the tubes were placed with the slanting surface downward. After this was done, the sporophores usually continued to develop normally, and in due season spores would be formed and discharged against the side of the tube opposite the hymenium.

The first sets of fungi which were kept in darkness were placed in pasteboard boxes in a horizontal position and these boxes inclosed in other pasteboard boxes. It was found, however, that some diffused light reached the tubes in spite of the double-box arrangement. Inside one of these boxes the recording tube of the soil thermograph was placed, while the registering portion of the instrument was kept on a shelf outside the boxes. These boxes were kept on the shelf beside the other boxes containing the tubes which were exposed to the direct rays of the sun. This was done in order to obtain as near as possible the same environment for the tubes kept in the sunlight and those kept in the darkness, except for the single factor of light.

In a later series of experiments, the ones which are recorded in detail in this paper, the cultures were kept in pasteboard boxes in a horizontal position and these boxes were placed in a photographic dark room from which all light was excluded by means of sheets of cardboard being placed over the ruby lights. By this arrangement absolute darkness was obtained for the cultures. The soil thermograph was arranged in the same manner in the dark room as when on the outside. Table XV shows the highest maximum and minimum, the lowest maximum and minimum, the highest and the lowest mean, and the average of the daily maximum, minimum, and mean temperatures for each month as recorded by the two thermographs.

TABLE XV.—*Temperature records (°F.) for cultures grown in daylight and in darkness*

Date and degree recorded.	In light.			In dark.		
	Maximum.	Minimum.	Mean.	Maximum.	Minimum.	Mean.
December 18–31, 1916:						
Highest	80	66	73	75	65	69
Lowest	72	55	65	66	57	64
Average	76	61	68	71	61	66
January, 1917:						
Highest	89	70	77	78	73	74
Lowest	65	52	58	63	58	60
Average	79	60	69	71	68	69
February, 1917:						
Highest	97	67	81	86	72	78
Lowest	74	56	66	68	59	65
Average	87	60	73	77	65	71
March, 1917:						
Highest	96	72	80	88	80	83
Lowest	71	50	62	73	62	68
Average	86.5	59	72.7	79	68	73
April, 1917:						
Highest	96	66	77	86	80	80
Lowest	69	46	65	68	65	67
Average	82	59	70.5	78.7	70.8	74.7
May, 1917:						
Highest	100	67	83	77	74	75
Lowest	61	49	56	60	57	59
Average	80	60	70	71	68	69.5
June, 1917:						
Highest	110	78	93	92	88	90
Lowest	82	50	68	70	69	70
Average	96	70	83	84	80	82

EXPLANATION OF TERMS USED IN SPOROPHORE TABLE XVI.

In Table XVI most of the headings are self-explanatory; however, there are a few that need special explanation. The heading for the sixth column is "Inoculum and date of inoculation." Under this heading the character of the inoculum used when sphorophores were produced is given, as well as the date or dates when each tube which produced sphorophores was inoculated. "Wood" means the inoculum used was infected wood; "tissue" means pieces of sphorophore tissue, while "malt," "potato," etc., signifies that mycelium was used as an inoculum and that it was taken from a culture on malt agar, potato agar, etc. "Inoculum as used means the piece of wood, tissue, etc., used to inoculate the media. "Development period of sporophores" means the number of days from the time the inoculation was made to the first evidence of the formation of a hymenium.

Table XVI shows that the authors have obtained under the cultural methods described 629 sporophores in the light and 11 in darkness, 640 in all, representing 4 families (Agaricaceae, Polyporaceae, Thelephoraceae, and Tremellaceae), 16 genera (Coprinus, Daedalea, Exidea, Fomes, Ganoderma, Irpex, Lentinus, Lenzites, Merulius, Panus (?) Pleurotus, Polyporus, Polystictus, Poria, Stereum, and Trametes), 42 species, and 97 strains from 65 host species collected in 11 States. If the genera given in North American Flora are used, there would be 24 genera, since the following 8 genera would be added: Coriollelus, Elfvingia, Inonotus, Laetiporus, Pycnoporus, Pyropolyporus, Spongipellis, and Tyromyces.

TABLE XVI.—*Data relating to sporophore production on artificial media*

Forest Pathology No.	Name of fungus.	State where collected.	Host.	Source of initial inoculum.	Inoculum and date of inoculation.	
					In light.	In dark.
21831	*Coprinus atramentarius*	Montana	*Populus trichocarpa*	Mycelium	Tissue, May 2, 1917	
21862	*Daedalea juniperina*	New Mexico	*Juniperus pachyphloea*	Wood	Wood, May 12, 1917	
21592	*Daedalea juniperina*	Arizona	*Juniperus utahensis*	do	Prune, Mar. 16, 1917	Carrot, Apr. 7, 1917.
21874	*Exi'ea recisa*	New Mexico	*Quercus gambelii*	do	Wood, May 24, 1917	
21830	*Fomes applanatus*	Colorado	*Populus trichocarpa*	Tissue	Tissue, May 29, 1917	
21602	*Fomes arctostaphyli*	Arizona	*Arctostaphylos pungens*	Wood	Wood, Oct. 24, 1916	Malt, Dec. 18, 1916.
21776	*Fomes laricis*	do	*Pinus ponderosa*	do	Prune, Mar. 14, 1917	
21756	*Fomes meliae* (?)	Texas	*Melia azedarach*	do	Wood, Jan. 11, 1917	Potato, Apr. 7, 1917.
21838	*Fomes pinicola*	Idaho	*Larix occidentalis*	Tissue	Parsnip, June 7, 1917	
21488	do	New Mexico	*Pseudotsuga taxifolia*	do	Malt, Dec. 20, 1916	Malt, Dec. 20, 1916.
21817	*Fomes rimosus*	Texas	*Acacia roemeriana*	Wood	Wood, Apr. 10, 1917	
21687	do	do	*Prosopis juliflora*	Tissue	Corn meal, June 2, 1917	
21741	do	do	*Siderocarpos flexicaulis*	do	Prune, June 1, 1917	
21611	do	Arizona	*Juglans rupestris* (?)	do	Tissue, Apr. 7, 1917	
21884	*Fomes robiniae*	New Mexico	*Robinia neomexicana*	do	Tissue and malt, May 24 and June 9, 1917.	
19114	*Fomes roseus*	do	*Populus tremuloides*	do	Tissue, May 26, 1917	
19534	do	do	*Picea parryana*	Wood	Wood, May 26, 1917	
21290	do	do	*Pinus edulis*	Tissue	Tissue, May 24, 1917	
21423	do	do	do	do	do	
21277	do	do	*Pseudotsuga taxifolia*	do	do	
21376	do	do	do	do	Tissue, May 26, 1917	
21835	do	Oregon	do	do	Tissue, May 2, 1917	
21880	*Fomes scutellatus*	New Mexico	*Quercus gambelii*	do	Tissue, May 24, 1917	
21880b	do	do	do	Wood	Wood, May 24, 1917	
21807	*Fomes texanus*	do	*Juniperus monosperma*	Tissue	Tissue Feb. 25, 1917; alfalfa, Mar. 12, 1917.	Prune, Apr. 7, 1917.
21653	do	do	do	do	Corn meal, Dec. 6, 1916, June 1, 1917.	Malt, Dec. 6, 1916, Apr. 7, 1917.
21806	do	do	*Juniperus scopulorum*	do	Tissue, Feb. 25, 1917	Prune, Apr. 7, 1917.
21760	do	do	do	do	Corn meal, Mar. 12, 1917	Corn meal, Apr. 7, 1917.
21729	*Ganoderma sessile*	Texas	*Quercus nigra*	do	Tissue, Jan. 27, 1917	Tissue, Jan. 27, 1917.
21775	*Irpex lacteus*	New Mexico	*Quercus arizonica*	Wood	Corn meal, Mar. 23, 1917	
21706	*Lentinus lepideus*	Texas	*Pinus palustris*	do	Wood, Nov. 25, 1916; prune, Mar. 12, 1917.	Wood, Nov. 25, 1916; corn meal, Apr. 7, 1917.
21587	*Lenzites saepiaria*	Arizona	*Pinus ponderosa*	do	Corn meal, Dec. 20, 1916	Corn meal, Dec. 20, 1916.
21878	do	do	do	do	Wood, May 24, 1917	
21794	*Merulius ambiguus*	New Mexico	*Pinus edulis*	do	Prune, Apr. 16, 1917; wood, Feb. 12, 1917.	Prune, Apr. 7, 1917.
21768	*Panus* sp. (?)	do	*Abies concolor*	do	Malt, Mar. 14, 1917	Alfalfa, Apr. 7, 1917.
21871	*Pleurotus ostreatus*	Colorado	*Populus* sp.	Tissue	Tissue, May 17, 1917	

TABLE XVI.—*Data relating to sporophore production on artificial media*—Continued

Forest Pathology No.	Name of fungus.	State where collected.	Host.	Source of initial inoculum.	Inoculum and date of inoculation.	
					In light.	In dark.
21747	*Pleurotus ostreatus*	New Mexico	*Populus wislizeni*	Tissue	Tissue, Dec. 28, 1916; corn meal, Jan. 6, 1917; spores, Jan. 17, 1917.	Bean, Jan. 17, 1917; spores, Apr. 7, 1917.
21586	*Polyporus* sp	Arizona	*Populus tremuloides*	do	Malt, Dec. 20, 1916, Apr. 17, 1917.	Malt, Dec. 20, 1916, Apr. 7, 1917.
21917	do	Texas	*Prunus persica*	Wood	Wood, June 8, 1917	
21919	do	New Mexico	*Acer negundo*	do	Wood, June 26, 1917	
21785	*Polyporus albidus*	Idaho	*Pinus ponderosa*	Tissue	Tissue, Feb. 2, 1917; potato, Apr. 18, 1917.	
21800	*Polyporus anceps*	do	do	do	Tissue, Jan. 29, 1917; potato, Apr. 17, 1917.	
21801	do	do	*Thuja plicata*	do	Tissue, Jan. 29, 1917; prune, Apr. 17, 1917.	
19867	*Polyporus cinnabarinus*	Arkansas	*Quercus alba*	Wood	Tissue, Mar. 9, 1917; malt, Feb. 14 and June 2, 1917.	Potato, Apr. 7, 1917.
21514	do	New Mexico	*Quercus gambelii*	do	Tissue, Feb. 14, 1917; potato, Mar. 9 and June 2, 1917.	Bean, Apr. 7, 1917.
21856a	do	do	do	do	Wood, May 12, 1917	
19001	do	Florida	*Quercus nigra*	do	Tissue, Feb. 14, 1917; malt, Mar. 9 and June 2, 1917.	Carrot, Apr. 17, 1917.
19793	do	Arkansas	*Quercus velutina*	do	Tissue, Feb. 7, 1917; prune, Mar. 9 and June 2, 1917.	Bean, Apr. 7, 1917.
21759	do	New Mexico	*Pinus edulis*	do	Tissue, Jan. 21, 1917; potato, Mar. 9 and June 2, 1917.	Potato, Apr. 7, 1917.
21484	*Polyporus dryophilus*	do	*Quercus gambelii*	do	Tissue, Jan. 29, 1917; prune, Mar. 10, 1917.	Prune, Apr. 7, 1917.
21789	do	Arizona	do	do	Corn meal, Mar. 9, 1917	Corn meal, Apr. 7, 1917.
21936	do	do	do	Tissue	Tissue, July 25, 1917	
21964	do	do	do	do	Tissue, Aug. 18, 1917	
21938	do	New Mexico	*Quercus arizonica*	do	Tissue, July 21, 1917	
21825	*Polyporus ellisianus* (?)	Idaho (?)	*Abies grandis*	do	Tissue, Feb. 2, 1917; potato, Apr. 17, 1917.	
21272	do	New Mexico	*Pinus edulis*	do	Tissue, Feb. 13, 1917	
21795	do	do	do	do	Corn meal, Feb. 13, 1917	
21352	do	Arizona	*Pinus ponderosa*	do	Tissue, Feb. 13, 1917; malt, Apr. 18, 1917.	Malt, Apr. 18, 1917.
21305	do	do	do	Wood	Corn meal, Jan. 9, 1917	Corn meal, Jan. 9, 1917.
21304	do	do	do	do	Corn meal, Jan. 9, 1917	Corn meal, Jan. 9, 1917.
19596	do	New Mexico	do	Tissue	Tissue, Feb. 13, 1917; prune, Apr. 17, 1917.	
21787	do	Arizona	do	Wood	Corn meal, Apr. 18, 1917	

21752	....do	New Mexico	....do	....do	Wood, Jan. 20, 1917; corn meal, Apr. 18, 1917.	
21750	....do	....do	....do	....do	....do	
21623	....do	Arizona	....do	Tissue	Tissue, Feb. 12, 1917; prune Apr. 17, 1917.	
21578	....do	....do	....do	Wood	Wood, Oct. 14, 1916; malt, Dec. 20, 1916, Apr. 18, 1917.	Corn meal, Dec. 20, 1916.
21910	....do	Montana	....do	Tissue	Tissue, May 18, 1917	
21591	*Polyporus farlowii*	New Mexico	*Acer negundo*	....do	Tissue, Mar. 9, 1917; parsnip, Apr. 7, 1917.	Parsnip, Apr. 7, 1917.
21402	....do	....do	*Populus italica*	....do	Tissue, Aug. 24, 1916; potato, Sept. 15, 1916, Apr. 7, 1917.	Potato, Apr. 7, 1917.
12986	*Polyporus obtusus*	North Carolina	*Quercus velutina*	Wood	Wood, May 24, 1917	
21679	*Polyporus sulphureus*	Texas	*Quercus virginiana*	Tissue	Chlamydospores from malt, Dec. 20, 1916, Mar. 15, 1917.	Dec. 20, 1916.
21201	*Polyporus texanus*	....do	*Prosopis juliflora*	....do	Prune, May 29, 1917	
21805	....do	....do	....do	Wood	Celery, June 1, 1917	
21891	*Polystictus hirsutus*	New Mexico	*Prunus melanocarpa*	....do	Wood, May 24, 1917	
21886	....do	....do	*Quercus gambelii*	....do	....do	
21906	....do	....do	....do	....do	....do	
21893	....do	....do	....do	Tissue	Tissue, May 24, 1917	
21908	....do	....do	*Acer negundo*	Wood	Wood, May 24, 1917	
21901	*Polystictus versicolor*	....do	*Quercus gambelii*	....do	Wood, May 24, 1917	
21872	....do	....do	....do	Tissue	Tissue, May 24, 1917	
21468	*Poria* sp	....do	*Juniperus pachyphloea*	....do	Tissue, May 12, 1917	
21580	....do	Arizona	*Pinus ponderosa*	....do	Tissue, Oct. 4, 1916; malt, Dec. 20, 1916.	Malt, Dec. 20, 1916; Apr. 7, 1917.
29873	....do	New Mexico	....do	Wood	Wood, May 24, 1917	
21707	....do	Texas	*Taxodium distichum*	....do	Wood, Nov. 25, 1916	Wood, Nov. 25, 1916; Malt, Apr. 16, 1917.
21397	*Stereum umbrinum*	Arkansas	*Quercus alba*	....do	Corn meal, Apr. 16, 1917	Corn meal, Apr. 16, 1917.
19905	....do	....do	....do	....do	Corn meal, Mar. 16, 1917	Potato, Apr. 7, 1917.
19895	....do	....do	....do	....do	....do	Bean, Apr. 7, 1917.
19783	*Stereum versiforme*	....do	....do	....do	....do	Corn meal, Apr. 7, 1917.
21870	*Trametes peckii*	Colorado	*Populus* sp	Tissue	Tissue, May 12, 1917; Carrot, June 7, 1917.	
21948	....do	New Mexico	*Populus angustifolia*	Spores	Spores, Aug. 9, 1917	
21948	....do	....do	....do	Tissue	Tissue, Aug. 17, 1917	
21792	....do	....do	*Populus wislizeni*	....do	Tissue, Feb. 12, 1917	
21791	....do	....do	....do	Wood	Spores, Mar. 12, 1917; Malt, June 7, 1917.	Celery, Apr. 7, 1917.
21761	*Trametes serialis*	....do	*Juniperus monosperma*	Tissue	Tissue, Jan. 15 and Mar. 13, 1917; prune, June 2 and June 9, 1917.	Parsnip, Apr. 7, 1917.
21868	....do	....do	*Juniperus pachyphloea*	Wood	Wood, May 12, 1917; Prune, June 7, 1917.	
21861	....do	....do	....do	....do	Wood, May 12, 1917; Malt, June 7, 1917.	
21866a	....do	....do	*Juniperus scopulorum*	....do	....do	

Forest Pathology No.	Name of fungus.	State where collected.	Host.	Media producing sporophores.		Number of sporophores produced.		Development period for sporophores.		Color of hymenium.
				Light.	Dark.	Light.	Dark.	Light.	Dark.	
								Days.	*Days.*	
21831	*Coprinus atramentarius*	Montana	*Populus trichocarpa*	Malt, parsnip		2		25–31		
21862	*Daedalea juniperina*	New Mexico	*Juniperus pachyphloea*	Corn meal, malt, prune.	o	6		8–17		White.
21592	*D. juniperina*	Arizona	*Juniperus utahensis*	Corn meal	o	4	o	20–21	o	Do.
21874	*Exidea recisa*	New Mexico	*Quercus gambelii*	Carrot, corn meal, prune, malt.		6		15–30		Auburn to carob brown.
21830	*Fomes applanatus*	Colorado	*Populus trichocarpa*	Malt, parsnip		3		30–38		White.
21602	*Fomes arctostaphyli*	Arizona	*Arctostaphylos pungens*	Malt	o	1	o	77	o	Buckthorn brown.
21776	*Fomes laricis*	do	*Pinus ponderosa*	Corn meal		1		85		White.
21756	*Fomes meliae* (?)	Texas	*Melia azedarach*	Corn meal, prune	o	2	o	28	o	White to light buff.
21838	*Fomes pinicola*	Idaho	*Larix occidentalis*	Prune	o	1	o	43	o	White.
21488	do	New Mexico	*Pseudotsuga taxifolia*	Corn meal	o	2	o	77	o	Do.
21817	*Fomes rimosus*	Texas	*Acacia roemeriana*	Carrot		2		52		Cinnamon brown.
21687	do	do	*Prosopis juliflora*	Malt	o	3	o	18	o	Do.
21741	do	do	*Siderocarpos flexicaulis*	do	o	1	o	18	o	Do.
21611	do	Arizona	*Juglans rupestris* (?)	Prune	o	3		15–33		Do.
21884	*Fomes robiniae*	New Mexico	*Robinia neomexicana*	Corn meal, prune		5		30–40		Buckthorn brown.
19114	*Fomes roseus*	do	*Populus tremuloides*	Carrot, parsnip		3		29		Pale cinnamon pink.
19534	do	do	*Picea parryana*	Malt, carrot, corn meal, parsnip, prune.		9		11–37		Shell pink to pale cinnamon pink.
21290	do	do	*Pinus edulis*	Carrot		3		21–40		Pale cinnamon pink.
21423	do	do	do	Prune, parsnip, malt, corn meal.		7		11–40		Shell pink to pale cinnamon pink.
21277	do	do	*Pseudotsuga taxifolia*	Corn meal, carrot, parsnip.		5		15–20		Pale pinkish cinnamon.
21376	do	do	do	Corn meal, carrot, prune, parsnip,		6		7–23		Do.
21835	do	Oregon	do	Prune	o	1	o	10	o	Shell pink.
21880	*Fomes scutellatus*	New Mexico	*Quercus gambelii*	Malt, prune, corn meal, parsnip, carrot.		7		9–15		White.
21880b	do	do	do	Carrot		2		16		Do.
21807	*Fomes texanus*	do	*Juniperus monosperma*	Malt, carrot	o	6	o	14–23	o	Buckthorn brown.
21653	do	do	do	Malt	o	8	o	16–22	o	Do.
21806	do	do	*Juniperus scopulorum*	do	o	1	o	18	o	Do.
21760	do	do	do	do	o	2	o	22–23	o	Do.
21729	*Ganoderma sessile*	Texas	*Quercus nigra*	Corn meal		1	o	30		
21775	*Irpex lacteus*	New Mexico	*Quercus arizonica*	Beet		1		88		Light buff.
21706	*Lentinus lepideus*	Texas	*Pinus palustris*	Corn meal		4		22–40		

21587	*Lenzites saepiaria*	Arizona	*Pinus ponderosa*	Malt, corn meal		9	0	20–31		Buckthorn brown.
21878	do	do	do	Corn meal, prune, parsnip.		3		19–32		Do.
21794	*Merulius ambiguus*	New Mexico	*Pinus edulis*	Malt, carrot, celery, bean, prune, corn meal, potato.		8	0	14–30	0	Pinkish cinnamon to orange cinnamon.
21768	*Panus* sp. (?)	do	*Abies concolor*	Corn meal, malt	0	12	0	57–62	0	White.
21871	*Pleurotus ostreatus*	Colorado	*Populus* sp	Malt		5		37		
21747	do	New Mexico	*Populus wislizeni*	Carrot, malt, beet, corn meal, prune, potato.	0	51	0	16–50	0	White.
21586	*Polyporus* sp	Arizona	*Populus tremuloides*	Malt, beet, prune, parsnip, carrot, corn meal.	0	14	0	15–23	0	Light buff to warm buff.
21917	do	Texas	*Prunus persica*	Corn meal		1		12		White.
21919	do	New Mexico	*Acer negundo*	Carrot, malt, parsnip, corn meal.		6		25–41		Cinnamon brown.
21785	*Polyporus albidus*	Idaho	*Pinus ponderosa*	Celery, carrot, bean, malt, potato, prune.		10		13		White to light buff.
21800	*Polyporus anceps*	do	do	do		13		5–33		Do.
21801	do	do	*Thuja plicata*	Carrot, celery, malt, bean, corn meal, prune, potato, parsnip.		15		5–16		Do.
19867	*Polyporus cinnabarinus*	Arkansas	*Quercus alba*	Malt, carrot, alfalfa.	0	8	0	17–33	0	Grenadine red to flame scarlet.
21514	do	New Mexico	*Quercus gambelii*	Malt, corn meal, potato, prune.	Malt	8	1	16–33	16	Do.
21856a	do	do	do	Carrot, malt, corn meal.		5		16–28		English red to grenadine red.
19001	do	Florida	*Quercus nigra*	Corn meal, malt	0	6	0	12–18	0	Grenadine red to flame scarlet.
19793	do	Arkansas	*Quercus velutina*	Carrot, celery, malt.	0	7	0	11–22	0	Do.
21759	do	New Mexico	*Pinus edulis*	Malt, celery, prune, potato, carrot, parsnip.	0	6	0	16–33	0	Pores orange chrome to flame scarlet.
21484	*Polyporus dryophilus*	do	*Quercus gambelii*	Corn meal, potato	0	3	0	10–11	0	Buckthorn brown.
21789	do	Arizona	do	Corn meal, prune, malt.	0	4	0	9–15	0	Do.
21936	do	do	do	Carrot, prune, beet		5		5–20		Do.
21964	do	do	do	Prune		2		7		Do.
21938	do	New Mexico	*Quercus arizonica*	Carrot		1		6		Do.
21825	*Polyporus ellisianus* (?)	Idaho	*Abies grandis*	Carrot, malt, bean, corn meal, prune, potato.		10		8–12		White.
21272	do	New Mexico	*Pinus edulis*	Malt, prune		5		4–5		White to light buff.
21795	do	do	do	Prune		1		41		White.

Forest Pathology No.	Name of fungus.	State where collected.	Host.	Media producing sporophores.		Number of sporophores produced.		Development period for sporophores.		Color of hymenium.
				Light.	Dark.	Light.	Dark.	Light.	Dark.	
								Days.	*Days.*	
21352	*Polyporus ellisianus* (?)	Arizona	*Pinus ponderosa*	Malt, corn meal, prune, celery, potato, carrot.	0	12	0	8–32	0	White to light buff.
21305	do	do	do	Malt, corn meal	0	6	0	32–34	0	Do.
21304	do	do	do	do	0	5	0	24	0	Do.
19596	do	New Mexico	do	Malt, prune, corn meal, potato.		11		8–32		Do.
21787	do	Arizona	do	Celery, corn meal		2		11		White.
21752	do	New Mexico	do	Malt, corn meal, prune.		6		25–47		Do.
21750	do	do	do	Malt, corn meal, parsnip.		7		22–59		White to warm buff.
21623	do	Arizona	do	Malt, prune		4		4–25		White to light buff.
21578	do	do	do	Malt, corn meal, prune, bean.	0	26	0	12–24	0	Do.
21910	do	Montana	do	Corn meal		2		4		White to cream buff.
21591	*Polyporus farlowii*	New Mexico	*Acer negundo*	Malt, carrot, corn meal, prune, corn beet.	Malt, ear prune.	12	6	13–38	15–20	Buckthorn brown
21402	do	do	*Populus italica*	Malt, corn meal, prune, beet, parsnip.	Corn meal, pune.	29	3	13–28	20–26	Do.
12986	*Polyporus obtusus*	North Carolina	*Quercus velutina*	Prune, carrot, corn meal.		4		30–36		Warm buff.
21679	*Polyporus sulphureus*	Texas	*Quercus virginiana*	Celery, prune	0	4	0	60	0	Pale orange-yellow
21201	*Polyporus texanus*	do	*Proposis juliflora*	Carrot		1		39		Clay color.
21805	do	do	do	Carrot, parsnip		3		37–68		Do.
21891	*Polystictus hirsutus*	New Mexico	*Prunus melanocarpa*	Carrot, prune, parsnip.		5		9–10		Light buff to warm buff.
21886	do	do	*Quercus gambelli*	Malt, carrot, corn meal, prune, parsnip.		10		9–12		Do.
21906	do	do	do	Malt, prune, carrot, corn meal, parsnip.		10		8–16		Do.
21893	do	do	do	do		9		8–27		Do.
21908	do	do	*Acer negundo*	Malt, prune, corn meal, carrot.	0	8		11–25		Do.
21901	*Polystictus versicolor*	do	*Quercus gambelii*	Parsnip		1		16		White.
21872	do	do	do	Malt		1		14	0	Do.

21468	*Poria sp.*	....do....	*Juniperus pachyphlœa*	Carrot, prune.		3		7–10			Do.
21580	....do....	Arizona	*Pinus ponderosa*	Malt, corn meal.	0	8	0	20–33		0	Do.
29873	....do....	New Mexico	....do....	Carrot, malt, prune		4		21–25			Baryta yellow.
21797	....do....	Texas.	*Taxodium distichum.*	Malt	0	1	0	72		0	Cadmium orange.
21397	*Stereum umbrinum.*	Arkansas.	*Quercus alba.*	Corn meal, parsnip, potato, beet, bean, alsaïa.	0	6	0	11–30		0	Purple drab to brownish drab.
19905	....do....	....do....	....do....	Bean.	0	1	0	17		0	Purple drab.
19895	....do....	....do....	....do....	Malt, beet.	0	2	0	17–19		0	Cinnamon-drab to brownish drab.
19783	*Stereum versiforme.*	....do....	....do....	Corn meal, beet, parsnip.	0	6	0	25–48		0	Cinnamon-drab to ochraceous tawny.
21870	*Tramates peckii.*	Colorado.	*Populus sp.*	Carrot, prune, parsnip, bean.		5		7–17			Clay color to wood brown.
21948	....do....	New Mexico.	*Populus angustifolia.*	Carrot, malt.		12	0	10–18			Wood brown.
21948	....do....	....do....	....do....	Malt, carrot, corn meal, prune, parsnip.		8		2–6			Do.
21792	....do....	....do....	*Populus wislizeni.*	Prune.		4		16–27			Do.
21791	....do....	....do....	....do....	Malt, carrot, beet, prune, parsnip.		19	0	11–28		0	Do.
21761	*Tramates serialis.*	....do....	*Juniperus monosperma.*	Prune, carrot, malt, corn meal, celery, potato.	Prune.	18	1	8–16		17	Light buff.
21868	....do....	....do....	*Juniperus pachyphlœa.*	Malt, parsnip, prune.		5		7–12			Do.
21861	....do....	....do....	....do....	Parsnip, carrot, malt, celery, corn meal, bean, potato.		11		7–14			Do.
21866a	....do....	....do....	*Juniperus scopulorum.*	Malt, prune, carrot, corn meal, parsnip, beet, bean, celery, potato.		12		7–18			Light buff to warm buff.

Sporophores were produced when various kinds of inocula were used, such as wood, tissue, spores, or the mycelium from other cultures and on a great variety of artificial culture media. The wide range of genera and species covered by this investigation, together with the large number of sporophores produced, clearly proves that the sporophore production here reported is not an accident, but is a constant and permanent performance determined in the great majority of cases by the presence of light and to a very limited extent by the character of the substratum. Aeration and humidity are two other factors which also enter into sporophores production on artificial media.

INFLUENCE OF SUBSTRATUM ON CHARACTER OF HYMENIUM

The influence of the host or substratum on the character of the spore-bearing surface is well illustrated in the several strains of *Polystictus hirsutus* when grown in artificial cultures. In all of these strains the best developed and well-defined pore surfaces are produced on carrot, malt, and parsnip agars. On these the pore surface is fairly typical of that produced in nature, both as to size and color of pores. In the prune agar the pore surface is usually reduced in the majority of the tubes to a few scattering irpiciform spines. On some of the prune-agar tubes the spines are not flattened like those of an Irpex, but are round like those of a Hydnum. In the corn-meal tubes the hymenium in a large majority of cases is very similar to that produced in various species of the Thelephoraceae, being reduced to a smooth or slightly granular surface in which there are no definite pores. In all of the tubes (carrot, malt, prune, corn-meal, and parsnip agars) there is an enormous production of spores irrespective of the character of the hymenium. In fact, in some of the tubes the first evidence of any sporophore production is the deposit of spores on the opposite side of the culture tube when even a careful examination with a hand lens fails to show any signs of pores or spines.

The size, shape, and coloring of the pores and tubes produced in artificial cultures on many of the agars are practically identical with those found in nature for a given species. However, it often happens that in abortive sporophores on certain agars the coloring is not as pronounced as on agars where the sporophores reach their full development. In *Polyporus cinnabarinus*, for instance, the sporophores produced on potato agar are nearly white, while the same strain will produce the typical grenadine-red to flame-scarlet pores on malt.

TRUE PILEI IN ARTIFICIAL CULTURES

One of the most interesting facts brought out in this investigation was that in all of the thousands of cultures made with the hundreds of sporophores produced not a single one had a typical pileus, unless the fungus was a gill-bearing form (Agaricaceae), when the culture tubes were so placed that the slant faced the light in such a manner that its rays were

more or less at right angles to the agar surface where the hymenium was being developed.

At first it was believed that the absence of pilei was probably due to the fact that the tubes were left in a horizontal position and therefore pilei had no chance to develop. The same results were obtained when the tubes were placed vertically with the slant side facing the light as when placed horizontally. Very recently, however, the writers have devised a method by which small but otherwise typical pilei have been grown on artificial media in the test tubes. The method was as follows: In place of arranging the culture tubes so that the light fell directly on the slanted surface the tubes were placed in one of two positions (1) vertical in opaque boxes in such a manner that the rays of light would fall on the tops of and parallel to the tubes and none on the sides or bottom, and (2) nearly horizontal, with slanted surface of the agar turned downward but with the light again falling only on the tops and parallel with the tubes. Typical sporophores were produced by this means for *Polyporus dryophilus*, *P. hirsutus*, and *Fomes rimosus*, the only species tried so far. Whether this method will produce pilei with all species is not known. There are objections to both methods where one desires to obtain spores for plating in order to obtain individual spore colonies—viz, when the culture tubes are kept vertically the discharged spores fall on the agar rather than on the inner surface of the culture tubes and when the culture tubes are kept nearly horizontal with the slanted surface downward from the first the mycelium grows around the edges of the agar onto the glass, thereby covering the surface of the tubes where the spores will fall. There is also this further objection to both methods—viz, that the agar in drying separates from the glass tubes in a very irregular manner in place of from only the top surface as it does when the tubes are kept horizontal with the slanted surface uppermost.

INFLUENCE OF LIGHT ON THE FORMATION OF PILEI

The pilei of the Polyporaceae always developed in such a manner that their tops were directly toward the sunlight. Also the pilei of the Agaricaceae when grown in cultures were strongly proheliotropic from the very beginning of their formation. This positive heliotropism was especially marked when the sporophores of *Lentinus lepideus* and *Pleurotus ostreatus* were developing. The writers tried *P. ostreatus* in three different positions: (1) Culture tubes placed vertically but with the lower part so shaded that the light entered at the top of the tube; (2) tubes placed horizontally and covered with black paper so that the light entered only at the top of the tube and this placed toward the light; (3) the third experiment was made in a flask in which the medium was slanted on the side of the flask. The flask was placed upright and the development of the sporophores from this more or less slanting surface was observed. In every instance from the earliest development of the

sporophore to the complete expansion of the pileus, the sporophores always pointed directly toward the light. In the case of the vertical tubes it was to be expected that normally these sporophores would grow vertically, since it was presumed that gravity as well as sunlight might be a factor in the upward growth of the sporophore. When the tube was placed horizontally, a totally different condition existed. In this case the sporophore developed directly toward the sunlight but at right angles to the force of gravity. In the experiment with the flask the upper two-thirds of the flask was covered with dark paper. Ten or fifteen sporophores developed on the slanting surface of the agar in the flask, all of them without exception pointing downward toward the source of light.

Lentinus lepideus was also grown on a more or less vertical slant with the face of the slant turned towards the light. Perfect sporophores which developed under these conditions always turned directly toward the sunlight.

INFLUENCE OF GRAVITY ON THE FORMATION OF PORES

In all of the experiments conducted with the Polyporaceae the formation of the pores was always parallel to the action of gravity. If the tubes were left in their original horizontal position with the slant side upward, pores developed on the slant with their mouths pointing upward, thus making them parallel to gravity. If, on the other hand, the culture tubes were placed in a vertical position, the pores were formed on the sides of the slant in such a manner that their mouths pointed more or less downward, or both upward and downward in some cases and parallel to the force of gravity. In fact, the position of the pores of a fungus which produces a vigorous sporophore in artificial cultures could be governed at will by simply changing the position of the tube in reference to gravity. It seems therefore that while light is usually the main factor governing the initiation of a hymenium, gravity is the dominant force which determines in what direction the pores will point, irrespective of the incident light or whether the spores when discharged will fall onto the walls of the pores or not.

INFLUENCE OF SUBSTRATUM ON PORE FORMATION

Although it is a well-known fact that cultures of strictly parasitic fungi can fruit on a wide range of media irrespective of the special host on which the parasite usually thrives, the idea seems to have been generally accepted that some special culture media would have to be used in order to obtain sporophores of the Polyporaceae on artificial media in test tubes, flasks, etc. The experiments here given show that the same general rule as to food material applies to the wood-rotting fungi as a whole—viz, that no special decoction is necessary for each species in order to obtain sporophores; in fact, the wide range of artificial media upon which many of the Polyporaceae have fruited is rather remarkable when the nature of these fungi is taken into consideration.

Table XVI shows that there were 56 strains of wood-rotting fungi which produced sporophores on malt agar, 52 on corn meal, 48 on prune, 44 on carrot, 27 on parsnip, 14 on potato, 11 on celery, 11 on beet, 10 on bean, and only 1 on alfalfa agar, while there are four of these agars preeminently suitable for sporophore production—viz, malt, corn meal, prune, and carrot, in the order given.

POSITION OF SPOROPHORES ON MEDIA

One most interesting fact in reference to sporophore production on artificial media is the fact that a very large percentage, probably more than 95 per cent, of the sporophores were developed on the upper half of the slant. No perfect sporophores have ever been observed by the writers other than on the slant, although the agar in the tubes often dries out sufficiently to leave ample room for the sporophores to develop from the slant to the bottom of the tube over a distance of from 60 to 80 mm. Whether this means that the formation of the sporophore was dependent upon a small amount of moisture or whether it needed the greater aeration which the upper end of the tube afforded is not known. In many instances, especially with the Stereums, the fruiting surface was formed in a narrow zone at the extreme upper limit of the agar slant. The studies so far made indicate that an important factor in this case is probably the drier condition of the agar at the upper end of the slant. It would seem that if the drying of the agar was the only requisite for the formation of sporophores that as the agar dries in the lower portion of the tube a condition would be reached which would normally produce sporophores; yet such is not the case. The by-products produced by the fungus may be more or less deleterious to the formation of sporophores; and, since such by-products would be more abundant in the lower portion of the tube than under the slant, it follows that few, if any, sporophores would develop there.

DENSITY OF MYCELIUM AND SPOROPHORE PRODUCTION

Another very peculiar fact develops that the sporophore production usually occurs on that portion of the agar slant where the aerial growth of mycelium is the least. If a dense mass of mycelium forms over the entire agar slant, the chance for the formation of pores on such a surface is materially lessened. This may explain to some extent why sunlight plays an important part in the production of sporophores, since cultures kept in the dark usually develop a denser mass of mycelium on the surface of the slant than corresponding cultures in the light. There are many species of fungi which have been tried in both light and darkness in which the aerial growth is very limited, even when grown in the dark; and still no sporophores were produced when in darkness, but were produced in the light.

INFLUENCE OF INOCULUM ON SPOROPHORE DEVELOPMENT

In a few of the species of fungi the interesting fact developed that when tissue from sporophores was used for the inocula the presence of this tissue materially shortened the development period for sporophores on the agar. For instance, in *Polyporus albidus* from *Pinus ponderosa* (FP 21875), when tissue was used as an inoculum on corn-meal agar, the development period of the sporophore was 5 days; when mycelium from potato agar was used the development period was 11 days; for *P. anceps* from *Thuja plicata* (FP 21801), when tissue was used on malt the development period for the sporophore was 5 days; when mycelium from potato agar was used for the inoculum on malt agar the development period was 12 days. This rapid formation of the sporophore when tissue was used as an inoculum was characteristic of many of the strains of this group of fungi.

This shortening of the development period of the sporophores is also found occasionally in other species of fungi than this group. For instance, it was rather marked in *Polyporus dryophilus* when fresh sporophore tissue was used, in various strains of *Trametes peckii*, and to a slight extent in *Fomes roseus*. In several cases not only was the development period for sporophores shortened by the presence of pieces of sporophore used as the inoculum, but cultures made from infected wood, mycelium or spores produced sporophores only on one or two agars, while the same species would produce sporophores on several media when the inocula were pieces of sporophores. In those cases where the sporophore tissue shortened the development period, the pores usually but not always start directly on the tissue inoculum and then spread rapidly often over the entire agar slant. In no case was the development of the pores limited to the pieces of inoculum, while in many instances the pores would start on areas not immediately adjacent to the inoculum.

SUMMARY

(1) The following criteria were found of value in the differentiation of the various species: (*a*) Macroscopic characters, including rapidity of growth, color of aerial and submerged mycelium, character of the aerial mycelium as to texture, etc., staining of the agar, decoloration of the agar, the comparative rate of growth between the aerial and submerged mycelium, etc. (*b*) Microscopic characters, such as septation, branching, size and color of hyphæ, clamp connections, polymorphism in spore formation, etc.

(2) The sunlight was found to accentuate the colors and tone down the mycelial growth of the fungus, thereby making it more characteristic and uniform for a given species than when placed under similar conditions in the darkness or in weak diffused light.

(3) The cultural characters of vegetative development of the various strains of a given species of fungus show no appreciable difference between cultures of this fungus whether obtained from infected wood or from

sporophores; neither do the hosts of the fungus seem to make any marked changes in the fundamental cultural characters when strains from different hosts are compared. There may be minor differences due to the host from which the strain came but nothing more.

(4) When the cultural characters of closely related but really distinct species are compared, marked and constant differences in the character of the mycelium will be found on certain corresponding agars in the series of cultures representing the two species, while if the two fungi are really the same species, no constant differences of specific rank will occur. Unknown rots can also be identified by making pure cultures of the causative organism from the diseased wood and determining from the cultural characters of the fungus thus isolated its identity.

(5) The presence of light is essential to the production of sporophores when grown on artifical media in the great majority of fungi here investigated, while the character of the substratum plays only a very minor roll in sporophore initiation.

(6) The medium on which the fungus is grown often governs to some extent at least the form of the hymenium which develops.

(7) The size, shape, and color of the pores and tubes produced in artificial cultures on many of the agars are practically identical with those found in nature for a given species.

(8) The pilei of both the Poplyporaceae and the Agaricaceae when grown in pure cultures on artificial media are from the very beginning of their formation strongly proheliotropic, while the formation of the pore tubes in the Polyporaceae is always such that they are placed parallel to the action of gravity.

(9) In a few species of fungi the presence of tissue as the inoculum shortened the period of sporophore development from one to several days.

(10) Workers with wood-rotting fungi now have the following means for determining the identity of a given fungus or the causative organism of a given rot: (*a*) The sporophore characters as usually found in nature, (*b*) the characters of the rot produced, (*c*) the vegetative characters developed when grown in pure cultures on artificial media when exposed to light, and (*d*) the characters of the sporophores and various spore forms when produced on artificial media.

LITERATURE CITED

(1) BREFELD, Oscar.
1877. BOTANISCHE UNTERSUCHUNGEN ÜBER SCHIMMELPILZE. III. BASIDIOMYCETEN I. 226 p., 11 pl. Leipzig.

(2) ———
1888. UNTERSUCHUNGEN AUS DEM GESAMMTGEBIETE DER MYKOLOGIE. VII. BASIDIOMYCETEN. II. PROTOBASIDIOMYCETEN. 178 p., 11 pl. Leipzig.

(3) ———
1889. UNTERSUCHUNGEN AUS DEM GESAMMTGEBIETE DER MYKOLOGIE. VIII. BASIDIOMYCETEN. III. AUTOBASIDIOMYCETEN. 305 p., 12 pl. Leipzig.

(4) BULLER, A. H. R.
1909. RESEARCHES ON FUNGI. 287 p., 83 fig., 5 pl. London, New York, [etc.]
(5) FALCK, Richard.
1902. DIE CULTUR DER OIDIEN UND IHRE RÜCKFÜHRUNG IN DIE HÖHERE FRUCHTFORM BEI DEN BASIDIOMYCETEN. *In* Beitr. Biol. Pflanz., Bd. 8, Heft 3, p. 307–346, pl. 12–17.
(6) ———
1909. DIE LENZITES-FÄULE DES CONIFERENHOLZES. *In* Möller, A. Hausschwamm-Forschungen. Heft 3, 234 p., 24 fig., 7 pl. Jena.
(7) ———
1912. DIE MERULIUS-FÄULE DES BAUHOLZES. *In* Möller, A. Hausschwamm-Forschungen. Heft 6, 405 p., 73 fig., 17 pl. Jena.
(8) HUMPHREY, C. J., and FLEMING, Ruth M.
1915. THE TOXICITY TO FUNGI OF VARIOUS OILS AND SALTS, PARTICULARLY THOSE USED IN WOOD PRESERVATION. U. S. Dept. Agr. Bul. 227 38 p., 4 pl. Bibliography, p. 37–38.
(9) LONG, W. H.
1917. A PRELIMINARY REPORT ON THE OCCURRENCE OF WESTERN RED-ROT IN PINUS PONDEROSA. U. S. Dept. Agr. Bul. 490, 8 p.
(10) ———
1917. THREE UNDESCRIBED SPECIES OF POLYPORES. *In* Papers New Mexico Chapter Phi Kappa Phi, v. 1, no. 1, p. 1–3.
(11) LYMAN, G. R.
1907. CULTURE STUDIES ON POLYMORPHISM OF HYMENOMYCETES. *In* Proc. Boston Soc. Nat. Hist., v. 33, no. 4, p. 125–209, pl. 18–26. Literature, p. 203–209.
(12) RUMBOLD, C.
1908. BEITRÄGE ZUR KENNTNIS DER BIOLOGIE HOLZZERSTÖRENDER PILZE. *In* Naturw. Ztschr. Forst. u. Landw., Jahrg. 6, Heft 2, p. 81–140, 6 fig., 1 pl. Literatur, p. 139–140.
(13) ZELLER, S. M.
1916. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. II. LENZITES SAEPIARIA FRIES, WITH SPECIAL REFERENCE TO ENZYME ACTIVITY. *In* Ann. Mo. Bot. Gard., v. 3, no. 4, p. 439–512, pl. 8–9. Bibliography, p. 504–509.
(14) ———
1917. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. III. PHYSICAL PROPERTIES OF WOOD IN RELATION TO DECAY INDUCED BY LENZITES SAEPIARIA FRIES. *In* Ann. Mo. Bot. Gard., v. 4, no. 2, p. 93–164, pl. 9–13, 11 charts. Bibliography, p. 154–155.

GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED

By W. A. Withers and Frank E. Carruth,

Chemical Division, North Carolina Agricultural Experiment Station

REVIEW OF PREVIOUS WORK

Since our previous publication (*17*)[1] on this subject, several articles have appeared in which other explanations of cottonseed-meal poisoning have been offered.

Thus, Rommel and Vedder (*14*) have suggested that poisoning by cottonseed meal is similar to beriberi, and is caused by deficient diets. This view was based on the similarity of post-mortem symptoms noted in pigs fed on rice and tankage.

Wells and Ewing (*15*) have concluded that cottonseed-meal injury is due in large part to incomplete diets.

Richardson and Green (*11*) fed white rats and concluded that cottonseed meal and flour are not actively toxic, but contain insufficient minerals and possibly inadequate amounts of the fat-soluble growth-promoting substance.

Osborne and Mendel (*10*) have secured results similar to those of the last-named authors with cottonseed meal and flour, but on subsequently feeding raw cottonseed kernels supplied by us, they have corroborated the results which we had obtained with the kernels. They admit the presence of a deleterious substance in raw cottonseed, but apparently still hold the view that cottonseed meal, the product resulting from cooking the kernels and pressing out the oil, is nontoxic, at least for rats and chickens (*9*).

Inasmuch as no comparative experiments with an isolated and purified substance have been reported, we present the results of additional experiments with various animals to supplement those given in our previous experiments, in which rabbits and fowls were used.

The toxic effect of an ether extract of raw cottonseed has been well shown in the rat-feeding experiments described by McCollum and coworkers (*6*) and by Osborne and Mendel (*10*). This extract contains about 2 per cent of gossypol, which is equivalent to about 0.6 per cent of the weight of the kernels from which the extract is obtained. Our rat diet, containing 20 per cent of this extracted oil, caused prompt decline in grown rats. Osborne and Mendel (*10*) used as little as 1 per

[1] Reference is made by number (italic) to "Literature cited," pp. 100-101.

Journal of Agricultural Research,
Washington, D. C.
lm

Vol. XII, No. 2
Jan. 14, 1918
Key No. N. C.—8

cent of the extract (equivalent to about 0.02 per cent of gossypol in the diet) and found that the growth of the rats was greatly retarded. Our experiments have led to the conclusion that raw cottonseed kernels are highly toxic to rats, but that cooked cottonseed is only slightly toxic. Whether cottonseed meal made from cottonseed sufficiently cooked with moist heat is toxic to rats seems to depend on the diet in which it is fed. In a short feeding experiment a diet such as was used by Richardson and Green (*11*) (45 per cent of cottonseed meal, 17 per cent of whole milk powder, 10 per cent of starch, and 28 per cent of lard) has shown no definite toxic effect [1] on our rats even when it contains a short-cooked (28 minutes) meal. When the meal is the sole source of vitamines, protein, and minerals, we have rarely had such favorable growth as is reported by Richardson and Green (*11*) and by Osborne and Mendel (*10*).

If compared with ether-extracted raw cottonseed or with soybean meal, the rate of growth has been very small. The explanation of this, according to Osborne and Mendel, might be that the diet was unpalatable and that consequently less food was ingested. Unpublished experiments indicate to us that there still remains something toxic in long-cooked cottonseed meals which in restricted diets is objectionable to rats, causing a lower food intake, but the effect of which is overcome in supplemented diets.

This seems to be the same phenomenon discussed by McCollum (7), who finds that in diets very well supplemented the toxic effect of the fat of wheat embryo and other slightly toxic substances is overcome. The evidence of a toxic factor of moderate intensity for rabbits and pigs is also furnished in our rabbit and pig experiments.

TOXICITY OF RAW COTTONSEED KERNELS

In a large number of experiments with rats we have found that the effect of cottonseed products on rats can be predicted accurately when chemical tests indicating the presence or absence of gossypol have been made (Table I). In order to appreciate the significance of some of the experiments we may briefly describe the properties of gossypol. A yellow plant pigment having the apparent formula $C_{30}H_{28}O_9$ (molecular weight 532), not soluble in and not extracted by petroleum ether, readily soluble in acetone and ether, moderately soluble in alcohol, benzene, and chloroform, dissolves readily in sodium hydroxid (NaOH) and sodium carbonate (Na_2CO_3) and is slowly soluble in sodium bicarbonate ($NaHCO_3$). It may be titrated as a dibasic acid with aqueous alkalies. It crystallizes well from a mixture of ether and acetic acid as a sparingly soluble substance containing 10.1 per cent (1 molecule) of

[1] Data in article not yet published.

acetic acid. When dissolved in ether or oil and treated with anilin, it forms a bright-yellow insoluble substance which is apparently a dianilin salt of gossypol (1 molecule of gossypol to 2 molecules of anilin) $C_{30}H_{28}O_8$, $2C_6H_5NH_2$. This substance is very insoluble in most solvents, even aqueous sodium hydroxid.

TABLE I.—*Results of feeding raw cottonseed kernels in milk diets to rats*

Diet.	Rat No.	Weight.			Period.	Remarks.
		Initial.	Final.	Change.		
		Gm.	*Gm.*	*Per cent.*	*Days.*	
Diet 366 (59 per cent of kernels, 17 per cent of whole-milk powder, 10 per cent of starch, 14 per cent of lard).	43	133	110	−17	7	Died 11th day.
Do	44	217	179	−17	7	Discontinued.
Do	45	156	128	−18	7	Recovered on ether-extracted kernels.
Do	46	139	109	−25	7	Do.
Diet 377 (30 per cent of kernels, 38 per cent of milk-powder, 11 per cent of starch, 14 per cent of lard, 7 per cent of butter).	71	154	100	−32	18	Died.
Do	72	186	112	−40	21	Do.
Diet 378 (28 per cent of dry-heated (110° C.) kernels, equivalent to 30 per cent of raw, 40 per cent of milk-powder; otherwise like diet 377).	74	130	87	−33	23	Do.
Do	75	117	80	−31	19	Do.
Diet 379 (10 per cent of kernels, 50 per cent of milk-powder, 12 per cent of starch, 28 per cent of lard).	58	160	107	−33	61	Do.
Do	59	170	112	−34	61	Do.
Do	60	131	103	−21	59	Discontinued.
Control diet 364 (60 per cent of milk-powder, 12 per cent of starch, 28 per cent of lard).	60	105	122	+16	20	Do.
Diet 367 (39 per cent of ether-extracted kernels, 17 per cent of milk-powder, 10 per cent of starch, 34 per cent of lard).	47	112	185	+65	85	Subsequently declined from disease.
Do	48	184	188	+ 2	85	Do.
Do	45	128	177	+38	14	Were on unextracted kernels for 1 week previous.
Do	46	104	159	+53	14	Do.

TABLE I.—*Results of feeding raw cottonseed kernels in milk diets to rats*—Continued

Diet.	Rat No.	Weight.			Period.	Remarks.
		Initial.	Final.	Change.		
		Gm.	*Gm.*	*Per cent.*	*Days.*	
Diet 368 (20 per cent of ether-extract (equivalent to about 60 per cent of raw kernels), replacing 20 per cent of lard in control diet 364).	51	139	112	−19	6	Died.
Do	52	125	101	−19	8	Discontinued.
Do	53	206	185	−10	8	Do.
Do	54	135	118	−12	8	Do.
Diet 369 (0.4 per cent gossypol (equivalent to about 20 per cent of ether extract), replacing 0.4 per cent of starch in control diet).	29	140	107	−23	8	Do.
Do	30	113	94	−16	4	Do.
Do	31	162	144	−11	4	Do.
Do	32	133	106	−20	7	Died.
Diet 373 (0.1 per cent of gossypol added to control diet).	58	141	102	−28	112	Discontinued.
Do	59	80	71	−11	108	Died.
Do	60	81	70	−13	112	Do.
The survivor, rat 58, was then put on the same diet minus gossypol.	58	102	138	+36	21	Discontinued.

The sparingly soluble compounds of gossypol with acetic acid and with anilin have been used to estimate the amount of gossypol present in cottonseed kernels. Both methods have given results which show that gossypol exists in cottonseed kernels to the extent of approximately 0.6 per cent.

In order to explain the change in toxicity in cottonseed after being cooked in the mill, we offer the following hypothesis: Under the action of moist heat the gossypol streams from the glands and is spread over the seed tissue. Part is oxidized to a less toxic substance which we may for convenience call "D-gossypol;" part is left in combination with the bases or protein as a salt of gossypol; and part is expressed in the oil. The degree to which these changes take place is dependent on the method of cooking and the condition of the seed. In dry heating to 100° C. there is practically no decomposition of the gossypol. In very dry seeds the gossypol may not spread over the seed tissue and be changed unless much moisture is added and the cooking prolonged.

Some quantitative data on the amount of gossypol left in the seed have been obtained from samples of kernels cooked various lengths of time—5, 10, 20, and 28 minutes. The percentage of gossypol extracted by ether in these cases was, respectively, 0.62, 0.24, 0.10, and 0.07.

METHOD OF REMOVING THE TOXIC SUBSTANCE FROM THE ETHER EXTRACT

By treatment of the ether extract of raw kernels with an excess of anilin the gossypol is practically quantitatively precipitated. The dianilin salt produced is extremely insoluble in most solvents except hot anilin and alcoholic potassium hydroxid (KOH). The substance itself is not toxic because of its insolubility. It passes through the alimentary canal unchanged, as can be seen by a glance at the feces. One-half gm. doses of this anilin compound were fed for seven consecutive days to a rabbit without result, and it was also given to rats in a milk diet (0.3 per cent). The food intake of the rats was not diminished; nor were the rats affected perceptibly

Gossypol "acetate" was then prepared from this compound as follows: The substance was decomposed by means of an alcoholic alkali. The anilin was steamed off, and the gossypol was extracted with ether and crystallized as the "acetate" by the addition of acetic acid. This was fed in amounts (0.25 per cent) equivalent to the anilin compound (0.3 per cent) fed previously. The rats which had not been affected by the anilin compound were promptly affected and consumed but little food (see Table II).

By passing steam through the extract from which the anilin compound had separated the excess anilin was removed, and the resultant oil did not prove toxic to rats. The result of this experiment has led us to believe that gossypol is the only substance in raw cottonseed possessing marked toxic properties. This conclusion was indicated in our previous experiments (*17*), wherein we found that the gossypol extract freed from gossypol was not toxic.

TABLE II.—*Results of feeding gossypol "acetate" to rats*

Diet.	Rat No.	Weight.			Period.	Remarks.
		Initial.	Final.	Change.		
		Grams.	*Grams.*	*Per cent.*	*Days.*	
Diet 436 (gossypol "acetate" (0.25 per cent) prepared from decomposition of the insoluble nontoxic gossypol-anilin compound, added to the control diet).	201	166	119	−28	12	Died.
Do	202	122	91	−25	12	Do.
Do	203	128	94	−26	14	Do.
Do	160	117	79	−32	14	Do.
Diet 448 (12.5 per cent ether extract freed from gossypol by treatment with anilin, replacing 12.5 per cent of lard in control diet).	171	150	190	+27	39	Alive.
Do	175	155	190	+25	39	Do.
Do	94	95	118	+24	39	Do.
Do	96	124	167	+35	39	Do.
Do	90	122	137	+12	39	Do.

TOXICITY OF GOSSYPOL TO RABBITS

In our previous paper (*17*) most of our feeding experiments with gossypol were with the "acetate," a crystalline substance containing acetic acid in its composition. This product had the same toxic action as the product precipitated by petroleum ether; therefore we inferred there was no change wrought by crystallization. We have recently fed gossypol in amounts equivalent to a toxic weight of cottonseed kernels and have found it to produce serious results in every case. Where gossypol itself is added to a diet in appreciable amounts, the toxic effect is marked.

Gossypol was mixed with the feed in four forms: (1) Precipitated (by petroleum ether), (2) recrystallized "acetate" (10.1 per cent of acetic acid), (3) "free" gossypol, a very pure product, and (4) as the sodium salt of gossypol (gossypol "acetate" neutralized with three molecules of sodium hydroxid, 10.5 c. c. of *N/2* alkali to 1 gm. of substance). The results are summarized in Table III.

TABLE III.—*Results of feeding gossypol to rabbits*

Diet.	Rabbit No.	Weight.			Quantity of gossypol eaten.	Equivalent in extracted kernels.	Feeding period.	Result.
		Initial.	Final.	Gain or loss.				
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Days.*	
Gossypol precipitated from ether solution by petroleum ether.	990	1,490	1,205	−285	1.81	200	24	Died.
Do	996	2,440	2,105	−335	2.61	290	26	Do.
Do	998	1,075	850	−225	.68	75	14	Do.
Recrystallized gossypol "acetate."	994	2,330	2,180	−150	.87	97	14	Do.
Do	1,001	975	820	−155	.47	53	8	Do.
Gossypol free from acetic acid (0.1 gm. daily per animal, equivalent to about 17 gm. of raw cottonseed kernels).	21	2,000	1,900	−100	0.4	.....	4	Died 15th day.
Do	22	1,500	1,440	− 60	.33	.....	4	Died.
Gossypol "acetate"	17	950	800	−150	.433	.....	.....	Do.
Do	18	850	750	−100	.333	.....	.....	Died 11th day.
Sodium salt of gossypol	19	700	590	−110	.153	.....	.....	Died 13th day.

A peculiar feature about the effect of gossypol and oftentimes of cottonseed kernels is that the animals may eat these substances for several days without being affected, then they may suddenly cease eating, waste away, and finally die. This was the case with rabbits 21 and 22 in this experiment.

In our previous paper (*17*) we described the nontoxic product obtained by oxidation of gossypol by action of air on its alkaline solution. This oxidation product may also be formed to some extent in the cooking of cottonseed, but there is no evidence of it. The meal still contains con-

siderable amounts (about 1 per cent) of a substance which we have called "D-gossypol."

"D-gossypol" is very slightly soluble in ether. For rabbits ether extraction does not render the meal nontoxic. But where we find in the meal after 6 hours' extraction with ether considerable amounts of a substance giving color reactions for gossypol or "D-gossypol," it would seem that the substance is bound in some way. To explain this we have assumed that it may be combined with the protein or some other constituent. There is some evidence of this in the properties of these substances. We have mentioned that gossypol combines with anilin and with acetic acid to form less-soluble compounds. Possibly similar combination may take place with free amino and free carboxyl groups in the protein molecule. Marchlewski (*8*) mentioned the fact that gossypol behaves like tannin toward basic dyes. Tannin also precipitates proteins as insoluble compounds. A similar combination of gossypol with protein may occur in the cooking of cottonseed.

Confirmatory evidence that these substances may be the cause of cottonseed-meal poisoning is given in the previous publications of this Station. Thus, when cottonseed meal is treated with an alcoholic alkali (*19*), the meal is rendered nontoxic to rabbits. When the meal is fed with iron salts to pigs (*18*) and rabbits (*16*), the toxic effect is greatly diminished. Pigs fed on cottonseed meal and corn meal (1 : 3) with ferrous sulphate (*copperas*) did not die in 180 days, whereas without ferrous sulphate all the animals died. Rabbits were fed 106 days with ferric ammonium citrate without harmful results. We have explained this by assuming that the alkali treatment promoted oxidation of the gossypol and by assuming that the iron salt formed an insoluble precipitate with the gossypol, or possibly assisted the organism to oxidize it.

The experiments referred to in this article support our previous view that gossypol is toxic and that it is the only toxic substance in the raw kernels.[1] Extensive experiments with various meals with rats, rabbits, fowls, and swine show that there still remains, even in thoroughly cooked meals, an injurious factor. Such thoroughly cooked meals are harmful to rabbits and swine, but seem to have little effect on rats and fowls when fed on adequate diets.

PRELIMINARY EXPERIMENTS WITH PIGS

In order further to test the correctness of our view that cottonseed-meal injury is due to a toxic substance rather than to dietary deficiencies, we have conducted a few preliminary experiments with small pigs.

It seemed desirable, in view of the extreme position taken by Rommel and Vedder (*14*) to ascertain (1) whether gossypol is toxic to pigs; (2)

[1] The results of other experiments showing that cooking exerts a profound influence on the toxicity will be published at an early date.

whether extraction of gossypol from cottonseed by a solvent renders the residue nontoxic; (3) whether by the addition of vitamine-containing feeds, cottonseed-meal poisoning can be averted.

Four small Duroc-Jersey pigs were confined in small pens about 3.5 by 8 feet. The pens had a concrete floor which was bedded with pine shavings. The water used was secured from the city mains. The pigs were fed the diets given in Table IV.

TABLE IV.—*Percentage composition of diets for pigs*

Feed.	Pig 1.	Pig 2.	Pig 3.	Pig 4.		
				Period 1 (1st–28th day).	Period 2 (29th–38th day).	Period 3 (39th–45th day).
Cottonseed meal	25	.....	.....	21.7	30	45.5
Corn meal	75	49.75	75	.....	.....	45.5
Gossypol	.....	0.25	.....	.....	.....	.....
Wheat bran	.....	50	.....	65	60	.....
Ether-extracted cottonseed kernels	.....	.....	25	.....	.....	.....
Milk (solids)	.....	.....	.....	13	10	9
Green feeds	.....	.....	.....	(a)	(a)	(a)

a About one-half pound daily.

Pig 2 received at the start 1.22 gm. of gossypol daily. This figure is based on yields (about 1 per cent) of crude crystalline gossypol acetate, obtained from "oil-free" cottonseed kernels. Gossypol "acetate" equivalent to the required amount of gossypol was dissolved in ether; the acetic acid present was removed from the ether solution by agitation with water. The ether solution of gossypol was then spread over a part of the corn meal and the ether evaporated. This was no doubt an unnecessary procedure, as we have found no difference in the action of the "acetate" and the "free" gossypol.

Pig 3 was fed on cottonseed kernels from which practically all the gossypol had been removed by percolation with ether.

Pig 4 was fed with a view to supplying any deficiency of vitamines in the cottonseed meal by wheat bran, whole milk, and some green food, chiefly leguminous. It is not possible to give the exact composition of the ration of this pig. The green feed (about ½ pound daily) was not always consumed. By disregarding the green feed eaten and by assuming that the milk contained 12 per cent of solids, the composition of the diet was approximately as given above.

Figures 1 and 2 and Tables V to VII summarize the important data of the experiment.

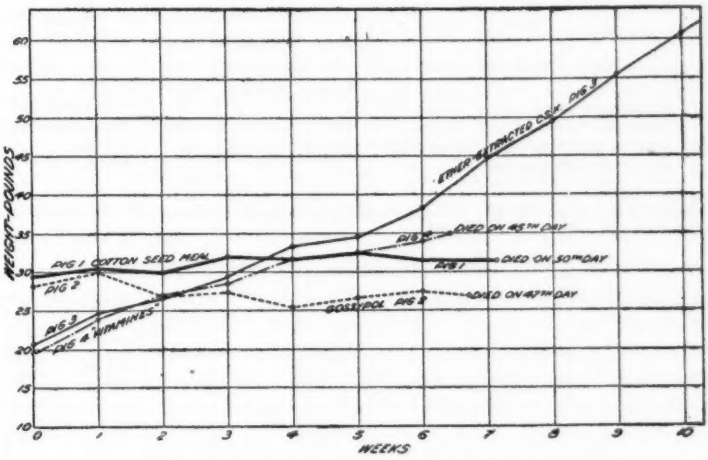


FIG. 1.—Graphs of the growth of pigs 1, 2, and 3. "C. S. K."=cottonseed kernels.

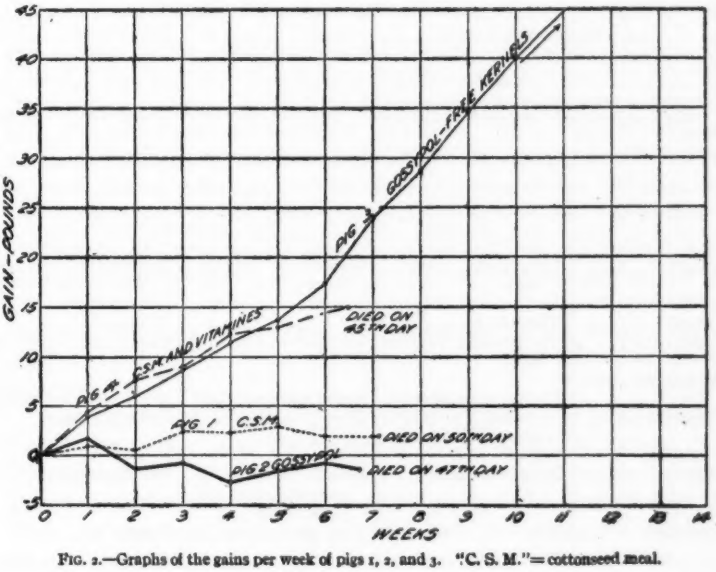


FIG. 2.—Graphs of the gains per week of pigs 1, 2, and 3. "C. S. M."=cottonseed meal.

TABLE V.—*Results of feeding various diets to pigs*

Pig No.	Feed.	Weight.			Result.
		Ihitial.	Final.	Gain.	
		Pounds.	*Pounds.*	*Pounds.*	
1	Cottonseed meal	29. 5	31. 5	2	Died 50th day.
2	Gossypol	28. 25	27	−1. 25	Died 48th day.
3	Ether-extracted cottonseed kernels	20. 75	[a] 44. 75	24	Lived.
4	Cottonseed meal, milk, etc	19. 5	35	15. 5	Died 45th day.

[a] Weight of pig 3 on the 50th day. This pig weighed 128 pounds when the experiment was discontinued on the 155th day, a daily gain of 0.69 pound.

TABLE VI.—*Weight of cottonseed feed consumed, by weeks* [a]

Week.	Pig 1 (cottonseed meal).	Pig 2 (gossypol).[b]	Pig 3 (extracted cottonseed kernels).	Pig 4 (cottonseed meal).
	Pounds.	*Gm.*	*Pounds.*	*Pounds.*
1	1. 68	8. 37 (1. 82)	1. 3	1. 3
2	1. 7	9. 76 (2. 15)	1. 5	1. 52
3	1. 63	8. 04 (1. 77)	1. 75	1. 43
4	1. 58	6. 76 (1. 50)	2. 18	1. 62
5	1. 36	4. 06 (. 90)	2. 18	2. 14
6	1. 27	5. 56 (1. 22)	2. 63	2. 8
7	1. 04	3. 50 (. 77)	2. 53	1. 1
Total cottonseed feed eaten	10. 26	49. 95 (10. 13)	14. 07	11. 91
Total feed eaten	41. 0	40. 5	56. 3	46. 0

[a] Maximum estimates are given for pigs 1, 2, and 4.
[b] Figures in parentheses give the weight (in pounds) of oil-free kernels, which correspond to the gossypol eaten.

TABLE VII.—*Comparison of post-mortem notes on pigs 1, 2, and 4*[a]

Organ, etc.	Pig 1.	Pig 2.	Pig 4.
Lungs	Congested, edematous.	Congested, edematous.	Extremely edematous, with some congestion.
Heart	Thrombus		Thrombus.
Chest cavity	2 to 3 ounces of serous fluid.	4 ounces of fluid	About 16 ounces of fluid.
Abdominal cavity	Slight excess of fluid.	Slight excess of fluid.	Slight excess of fluid.
Small intestines	Considerable injection of blood vessels.	Deeply injected	Inflamed areas.
Penis	Sheath swollen	Sheath swollen, organ paralyzed, and protruding.	Sheath swollen.
Nutrition	Poor	Very poor	Good.

[a] These pigs were examined by Dr. G. A. Roberts, Veterinarian of this Station.

GENERAL DISCUSSION

For the first few days of the experiment all the pigs ate well, and all gained in weight. Pigs 1 and 2 occasionally left part of their feed. In two weeks' time all except pig 3 began to show loss of appetite and regularly left a portion one-half to one-fourth of their feed. On the twenty-fifth day the pig 2 (fed gossypol) was quite sick and not able to walk well. At this time pig 3 was the thriftiest of the four, while the rations of No. 2 and 3 were reduced on account of refusal to eat. On the twenty-ninth day the ration of pig 4 was changed to 1 part of cottonseed meal and 2 parts of bran. On the thirty-second day the feed of pig 2 was changed. The wheat bran was replaced by middlings, for which the pig had a better appetite. On the thirty-ninth day the wheat bran in the ration of pig 4 was replaced by corn meal, the pig getting equal parts of cottonseed meal with corn meal. At that time this pig was leaving one-half to two-thirds of the wheat-bran mixture. For three or four days he ate the new mixture with much better appetite, but then refused a large part and died on the forty-fifth day. Pig 3 maintained perfect appetite up to the forty-ninth day, when she did not clean up the last trace of feed as usual. When removed to the yard to be photographed, it was noted that she had an abnormal gait in walking, the forelegs showing a tendency to double under her. This animal seemed to have a great desire to eat dirt, manure, etc. However, on being allowed the freedom of a large lot, the animal soon recovered. She was given a little ferrous-sulphate solution, chalk, and milk on the fiftieth day. Her normal appetite returned, and in three or four days she was able to trot. At no time did this pig show the rough coat and lack of appetite that characterized the others. On the fifty-second day the feed of cottonseed was increased slightly, and the pig received about one-half pint of milk daily for the following nine days. Whether this pig was suffering from deficiency of some sort in the ration, from lack of exercise, or from the daily intake of a small amount of gossypol in the kernels, we are not able to say. Even granting that this pig had a slight attack of beriberi, we can reasonably conclude from the experiment that the deficiency factor is one quite secondary to the toxicity factor. Plate 1 shows the condition of these pigs at various periods of the experiment.

This preliminary experiment was originally planned to run for a short period, but as the pig on the extracted kernels seemed to be in a pathological condition, in that the forelegs tended to double under her when she attempted to run, it seemed desirable to continue the animal on this diet. At this time, when pig 1, which had been given the diet most closely resembling that of pig 3 in chemical composition, had died in a rather emaciated condition, pig 3 was a plump, very-well nourished animal (Pl. 1, B). It is quite possible that the above-mentioned condi-

tion was due both to a slight toxicity of the extracted kernels and to the restricted diet. This condition was remedied, as previously described. The animal was continued on the outdoor turf lot for 35 days and then removed to a small indoor pen, where for 70 days longer she gained steadily.

Throughout the experiment the daily feed was maintained at 1 per cent (of body weight) of extracted kernels plus 3 per cent of corn supplement.

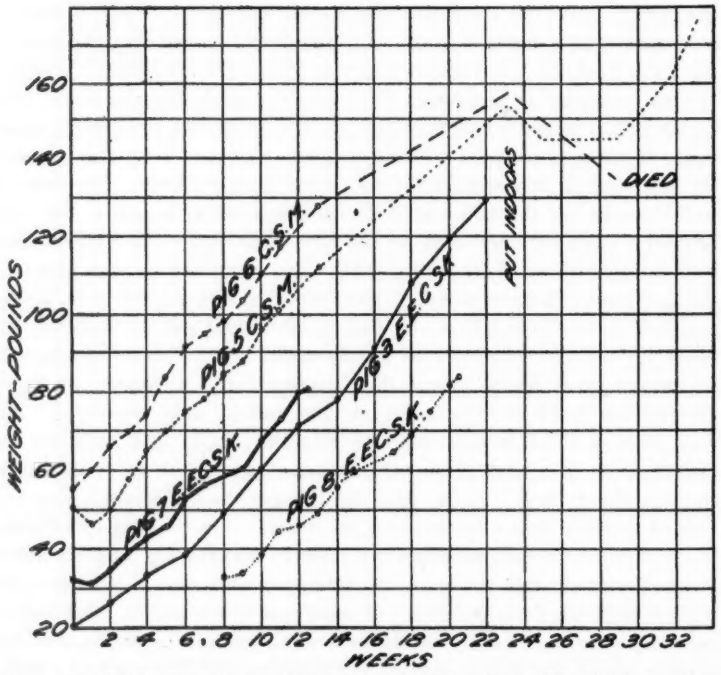


FIG. 3.—Graphs of the growth of pigs 3, 5, 6, 7, and 8. "C. S. M."=cottonseed meal: "E. E. C. S. K."=ether-extracted cottonseed kernels.

Two other small pigs, No. 7 and 8, were also fed on this diet of extracted kernels and corn meal on the same basis as in the previous case. On a few days it was necessary to use whole or cracked corn in place of corn meal, owing to a lack of meal. The results in general were the same. There was fair growth, but after six or eight weeks the pigs lost their keen appetites and developed a tendency to squat on their hindquarters and to walk stiffly. The growth curves in figure 3 show the general results. The gain was fair, 0.581 pound per day for the female and 0.556 pound for the male pig. The experiment was discontinued on the eighty-eighth day.

The female pig was observed for a few weeks after the experiment. The animal continued to increase in weight, but still retained the squat ting tendency. Addition of small amounts of milk and outdoor exercise did not eliminate this condition. The nutrition of the animal was excellent throughout.

In view of the slight toxicity of the extracted kernels, as shown in our rabbit experiments, it is possible that this slightly pathological condition may be due to a toxic factor, although with such very young pigs it may be due to the limitations of this food mixture.

No doubt if these pigs had been continued on this diet confined to pens, they would have ultimately failed, as do swine fed on restricted diet of cereal grains (see Hart and McCollum, *2*). This phenomenon, however, should not be confused with what is commonly understood as cottonseed-meal "injury" or poisoning.

We do not claim, however, that this diet is an adequate one, and it is quite possible that the condition described was due in large part to the inadequacy of certain dietary factors. Just what factors are insufficient in this particular diet is not at present apparent. In considering this question we have taken the view, tentatively, that the supply of vitamines in cottonseed is similar to that of other seeds and that the mineral content is very much better.

The fact that cottonseed is cooked, and subsequently pressed, raises the question, Are the vitamines thus rendered partially inactive or removed by the crude oil? In answer to this, we may point to the excellent growth of rats reported by Richardson and Green (*11*, *12*, *13*,) and Osborne and Mendel (*10*) as evidence that even the cooked meal is as well supplied with vitamines as any similar vegetable food.

With the dietary factors more favorable, as in the cottonseed-meal, experiment to be described, probably the pigs would not have manifested this stiffness of gait and squatting tendency. It is also of interest to compare the mineral content of a diet of cottonseed meal and corn meal (1:3) with the ash content of a diet found successful for growth in rats. (See Table VIII.)

TABLE VIII.—*Average mineral content of a diet containing 1 part of cottonseed meal and 3 parts of corn meal; also the analysis of minerals of other materials*

Feed.	Protein.	Ash.	Sodium.	Potassium.	Calcium.	Magnesium.	Chlorin.	Phosphorus.	Sulphur.
Cottonseed meal.....	36	7.0	0.26	1.66	0.27	0.55	0.04	1.35	0.49
Corn meal.....	87	.8	.113	.19	.015	.122	.07	.264	.20
Average mixture 1:3.	15.5	2.3	.14	.55	.08	.22	.055	51	.20
Pig and rat diet A (*2*).....		3.3	.022	.335	.266	.275	.041	.248	.089
Diet B (*3*).....		.63	.029	.076	.080	.009	.057	.186	.141
Dry skim milk (*1*)....		7.17	.488	1.27	1.34	.146	.935	.979	.357

Rat diet A represents the mineral content of a diet which was found successful for growth of pigs on artificial rations by Hart and McCollum (*2*).

Rat diet B represents the mineral content of a diet which did not produce pronounced stunting in rats (*4*). The diet was satisfactory in other factors.

The recent work of Hogan (*3*) and of McCollum, Simonds, and Pitz (*5*) indicates that the deficiencies of corn lie in the poor ash content, in poor proteins and low amounts of protein, and inadequate amounts of fat soluble A.

Since our pigs on ether-extracted kernels plus corn meal made much better gains than could be expected on whole corn alone under these conditions, our opinion is confirmed that the addition of this cottonseed feed to a corn diet furnishes a greatly improved protein and a mineral basis for nutrition. It is then evident that, where death ensues or poor growth is manifested, this is a result of an injurious substance rather than of dietary deficiencies.

FURTHER EXPERIMENTS WITH GOSSYPOL

Gossypol was fed to two other pigs of approximately 50 pounds' weight. In one case a rather large amount was given in the ration. Pig 5 ate a slop made of 1 pound of a mixture of corn meal and soybean meal (1:3) containing 4.5 gm. of gossypol. The pig showed a poor appetite for the same amount given the next morning, but ate it slowly after a pint of milk was poured into it. Next, a half dose (containing 2.3 gm.) was offered, one-half of which was refused. For the next five days the pig was offered smaller doses, but refused it all or in part, even when tempted to eat by using bran and milk in the feed. The pig showed a good appetite for other feed that did not contain the gossypol mixture. On the fifth, sixth, and seventh days the pig showed no appetite at feed time but seemed sluggish and showed a desire to lie down. The pig lost in weight. The experiment was then stopped. A control pig, No. 6, fed on a similar ration without gossypol showed a good appetite and developed no such symptoms.

After a rest of six days, the previous control pig, No. 6, was used for a gossypol experiment, and No. 5 served as the control. Gossypol was fed in amounts equivalent to that in 0.5 to 0.6 pound of oil-free cottonseed kernels, approximately 2.2 gm. daily. An ether solution of gossypol was dried on corn meal and this was mixed with more corn meal and wheat bran. The other pig received the same ration without gossypol. The gossypol pig ate practically all its feed for a week, and then began to show a poor appetite for it and refused part. On the eighth day one-half the feed was left. On the ninth and tenth days the pig ate scarcely any feed. From the eleventh to the fifteenth day the animal was given dry recrystallized gossypol acetate (10 per cent acetic

acid) as a finely crystalline powder mixed with the feed in place of gossypol evaporated on corn meal. The pig ate it for one day, and then refused it as before. By using corn meal only the animal ate one more feed, but refused it when repeated. During the last few days of the experiment the gossypol animal seemed to be growing weaker and very preceptibly thinner. In the last week the animal lost 3 pounds, while the control gained 2 pounds. This experiment was discontinued on the fifteenth day because of the refusal of the animal to eat.

In both of these cases of feeding gossypol it was very evident that the animals were physiologically affected at an early date. All told not over 15 gm. of gossypol were eaten in the first case and not over 22 gm. in the second case. These amounts of gossypol are equivalent to about 4 and 6 pounds of oil-free cottonseed kernels, respectively.

Besides the direct proof of the existence of a toxic substance in cottonseed meal, we have a strong argument against the deficiency theory in the results of feeding rabbits on cottonseed meal treated with boiling alcoholic alkali. This treatment, which would be expected to destroy the natural vitamines present, so changes the meal that it becomes nontoxic to rabbits. This change in toxicity we have shown is explained by the ease with which gossypol undergoes oxidation in alkaline solution. This was confirmed by feeding to rabbits the products formed by oxidation of gossypol in alkaline solution by air.

CAN COTTONSEED-MEAL POISONING BE OVERCOME IN A FAVORABLE DIET UNDER FARM CONDITIONS?

The two largest and oldest pigs (No. 5 and 6) were fed in a turf lot about 50 feet square. Grass was abundant, and a good part of the time there was water in the lot from frequent rains which also kept the turf soft. These pigs were fed on a mixture of equal parts of cottonseed meal, corn meal or corn, and wheat bran, with a pint of milk apiece each day. The cottonseed meal fed each day was about 1.33 per cent of body weight, rather higher than has been the practice at this Station. It was thought that for a while, about the fiftieth day, the pigs acted somewhat suspiciously. One showed a lack of appetite for the mixture. Its eyes seemed partly closed and somewhat watery. The pigs also seemed rather short-winded. They retained, however, perfect control of their limbs and were able to run very well at all times. Finally, after 160 days of high feeding of cottonseed meal, these pigs were put under cover in a small pen. The pigs were soon eating sparingly and losing weight. The pig which had acted somewhat queerly at times in the experiment became sick and died on the one hundred and ninety-eighth day, showing typical symptoms, although there was also a pneumonic appearance of the lungs. The experiment with the other pig was then discontinued. On changing this pig's feed to corn, he began to regain weight. This animal was later fed corn, wheat bran, meat scraps, etc. (Table IX.)

TABLE IX.—*Results of feeding cottonseed meal to pigs 5 and 6 under favorable conditions.*

Time of weighing.	Weight.			
	Pig 6.	Pig 5.	Gain per day.	
			Pig 6.	Pig 5.
Day.	*Pounds.*	*Pounds.*	*Pound.*	*Pound.*
Initial (June 28)	55.5	50.5	.....	.....
92	129	112.5	0.8	0.67
160	158	153.5	.64	.65
177	150	145	.....	.....
192	142	145	.....	.....
198 (Jan. 12)	[a] 135	(b)	.....	.....
217	.....	158	.....	.....
225–230	.....	[c] 177	.....	.....

[a] Dead. [b] Discontinued feeding cottonseed meal. [c] Slaughtered.

As long as these pigs were kept in the outdoor lot, no marked symptoms of cottonseed-meal poisoning were noted. It was noted that when kept indoors they lost both appetite and weight.

It is evident from our indoor and outdoor experiments that the effect of cottonseed meal is more severe on pigs kept in pens, a fact that has long been known; however, past records show that typical sudden deaths from "acute cottonseed-meal poisoning" may also occur among pigs receiving cottonseed meal when on pasture. Such deaths may follow excellent gains and may be without previous sickness, often occurring when the animals are exercised violently. Consequently, a conclusion that the meal was without effect during the outdoor experiment can not be drawn. Certainly the subsequent loss in weight after removal from the turf is suspicious. It is plausible to suppose that the outdoor conditions stimulate metabolism so that the animal is enabled to overcome or resist the injurious factor. Possibly the difference in effect of cottonseed meal on rats and pigs may be in part explained by the more vigorous metabolic activity of the smaller animal.

In a series of three recent articles, Richardson and Green (*11*, *12*, *13*) have well shown the high nutritive efficiency of cottonseed meal and flour for rats, indicating the economic value of this substance. In some points, however, we believe that they have misinterpreted facts. Thus, they speak of the flour as a "highly milled" or "refined" product and account for the apparent slight nutritive superiority of the unbolted meal over the flour by stating that—

> This suggests a greater amount of the growth-promoting substance associated with certain fats in the less highly milled product.

It seems hardly possible in the case of cottonseed meal to effect such a change in nutritive value by mechanical means. We would suggest that the difference was accountable on the basis of different conditions

in the cooking of the products, as we have found these to be the greatest cause of variation in toxicity of meals.

Richardson and Green (*11, p. 316*), state in conclusion:

> Our results indicate that cottonseed meal does not contain sufficient minerals for growth, is not actively toxic, contains efficient protein and perhaps fat-soluble, growth-promoting substances, similar to those of butter fat, but in less adequate quantities.

Our own extensive unpublished experiments on the toxicity of cottonseed products indicate that the toxicity of cottonseed meals varies with the conditions of cooking the raw seed. While we find that the flour and thoroughly cooked meals have no apparent toxicity for rats when fed in diets supplemented by milk powder, these same products fed in unsupplemented diets are inferior to ether-extracted cottonseed kernels. Even thoroughly cooked cottonseed meals are definitely injurious to rats and pigs.

The ash analysis of cottonseed flour given by Richardson and Green (*11, 12, 13*) in each of these three articles differs radically in some respects from that given by Forbes (*1*) (Table X).

TABLE X.—*Ash analyses of cottonseed flour*

Constituent.	Analysis of cottonseed flour according to Richardson and Green.	Constituent.	Analysis of cottonseed meal according to Forbes.
	Per cent.		*Per cent.*
Inorganic salts	5.50	Ash	7.629
Silicic oxid (SiO_2)	0.14		
Chlorin	None.	Chlorin	.042
Sulphur trioxid (SO_3)	.06	Sulphur	.536
Phosphorus pentoxid (P_2O_5)	2.57	Phosphorus	1.479
Potassium oxid (K_2O)	2.01	Potassium	1.81
Calcium oxid (CaO)	.26	Calcium	.291
Magnesium oxid (MgO)	.25	Magnesium	.599
Sodium oxid (Na_2O)	None.	Sodium	.283

Richardson and Green's data are from an analysis of material left after ignition, which, as is well known, causes loss of elements, such as sulphur and chlorin. While the elements chlorin and sodium are not necessary for plant growth and the amounts present may vary, it seems hardly possible that they are entirely lacking in cottonseed flour. They are certainly essential to animals. It may be noted that the rats of Richardson and Green "have grown and maintained body weight for 135 days" on a diet containing cottonseed flour as the sole source of minerals.

In their second article Richardson and Green (*12*) have attempted to repeat some of our work with extracts of cottonseed. Instead of using unheated cottonseed kernels, as we did, they used kernels heated to 120° C. Thus, they fail to find toxic the ether extract of petroleum-

ether-extracted kernels. Their results may be due to the influence of previously heating the kernels to 120° C., thus possibly decomposing some of the gossypol, and to incomplete extraction so that the remainder was left in the three fractions, the extracts and the residue. They also assume that the ethyl-ether extract of petroleum-extracted kernels is always 2 per cent of the weight of the kernels. This is the case only with long-continued extractions.

SUMMARY

Raw cottonseed kernels contain about 0.6 per cent of gossypol and are highly toxic to rats. Ether extraction renders the material nontoxic and gives a highly toxic extract containing about 2 per cent of gossypol. Gossypol fed in milk diets in amounts equivalent to those contained in the raw cottonseed diets has proved as toxic as raw cottonseed. Gossypol may be quantitatively removed from the ether extract by precipitation as its insoluble anilin compound. The extract is thus rendered nontoxic to rats. The insoluble anilin compound of gossypol is not toxic because of its insolubility. Gossypol prepared from this compound possesses its original toxic properties.

Cottonseed meal is much less toxic than raw cottonseed, owing mainly to the oxidation of gossypol during cooking.

Cottonseed meal, ether-extracted cottonseed, and gossypol have been fed to small pigs in pens under comparable conditions. Cottonseed meal has been found definitely injurious, while the ether-extracted raw seed does not appear to cause cottonseed-meal poisoning. Gossypol has been found toxic to pigs.

If the presence of an injurious substance in the meal is disregarded, a diet of cottonseed meal and corn meal has nutritive limitations which may, under restricted conditions of living, lead to failure of pigs to thrive. Such failure is a phenomenon distinct from cottonseed-meal poisoning.

Outdoor exercise, access to forage and soil, and improved diets tend to postpone or avert cottonseed-meal poisoning of swine. The deficiency hypothesis that cottonseed-meal poisoning of swine is similar to beriberi is untenable.

LITERATURE CITED

(1) Forbes, E. B., Beegle, F. M., and Mensching, J. E.
1913. MINERAL AND ORGANIC ANALYSES OF FOODS. Ohio Agr. Exp. Sta. Bul. 255, p. 211–231.

(2) Hart, E. B., and McCollum, E. V.
1914. INFLUENCE ON GROWTH OF RATIONS RESTRICTED TO THE CORN OR WHEAT GRAIN. *In* Jour. Biol. Chem., v. 19, no. 3, p. 373–395, 11 charts, 1 pl.

(3) Hogan, A. G.
1916. THE NUTRITIVE PROPERTIES OF CORN. *In* Jour. Biol. Chem., v. 27, no. 1, p. 193–208. Bibliography, p. 208.

(4) McCollum, E. V., and Davis, Marguerite.
1915. THE INFLUENCE OF THE COMPOSITION AND AMOUNT OF THE MINERAL CONTENT OF THE RATION ON GROWTH AND REPRODUCTION. *In* Jour. Biol. Chem., v. 21, no. 3, p. 615–643, 11 charts.

(5) ——— Simmonds, Nina, and Pitz, Walter.
1916. THE DIETARY DEFICIENCIES OF THE MAIZE KERNEL. *In* Jour. Biol. Chem., v. 28, no. 1, p. 153–165, 10 charts.

(6) ——— ——— ———
1916. THE DISTRIBUTION IN PLANTS OF THE FAT SOLUBLE A, THE DIETARY ESSENTIAL OF BUTTER FAT. *In* Amer. Jour. Physiol., v. 41, no. 3, p. 361–375, 2 fig., 11 charts.

(7) ——— ——— ———
1916. THE NATURE OF THE DIETARY DEFICIENCIES OF THE WHEAT EMBRYO. *In* Jour. Biol. Chem., v. 25, no. 1, p. 105–131, 19 charts.

(8) Marchlewski, L.
1899. GOSSYPOL, EIN BESTANDTEIL DER BAUMWOLLSAMEN. *In* Jour. Prakt. Chem. N. F., Bd. 60, Heft 1/2, p. 84–90.

(9) Osborne, T. B., and Mendel, L. B.
1916. THE EFFECT OF THE AMINO-ACID CONTENT OF THE DIET ON THE GROWTH OF CHICKENS. *In* Jour. Biol. Chem., v. 26, no. 2, p. 293–300, 1 pl.

(10) ——— ———
1917. THE USE OF COTTON SEED AS FOOD. *In* Jour. Biol. Chem., v. 29, no. 2, p. 289–317, 5 charts.

(11) Richardson, Anna E., and Green, Helen S.
1916. NUTRITION INVESTIGATIONS UPON COTTONSEED MEAL. I. *In* Jour. Biol. Chem., v. 25, no. 2, p. 307–318, 5 charts.

(12) ——— ———
1917. NUTRITION INVESTIGATIONS UPON COTTONSEED MEAL. II. *In* Jour. Biol. Chem., v. 30, no. 2, p. 243–258, 13 charts.

(13) ——— ———
1917. NUTRITION INVESTIGATIONS UPON COTTONSEED MEAL. III. COTTONSEED FLOUR. THE NATURE OF ITS GROWTH-PROMOTING SUBSTANCE AND A STUDY IN PROTEIN MINIMUM. *In* Jour. Biol. Chem., v. 31, no. 2, p. 379–388, 4 charts.

(14) Rommel, G. M., and Vedder, E. B.
1915. BERIBERI AND COTTONSEED POISONING IN PIGS. (PRELIMINARY NOTE.) *In* Jour. Agr. Research, v. 5, no. 11, p. 489–493.

(15) Wells, C. A., and Ewing, P. V.
1916. COTTONSEED MEAL AS AN INCOMPLETE FOOD. *In* Jour. Biol. Chem., v. 27, no. 1, p. 15–25. References, p. 24–25.

(16) Withers, W. A., and Brewster, J. F.
1913. STUDIES ON COTTONSEED MEAL TOXICITY. II. IRON AS AN ANTIDOTE. *In* Jour. Biol. Chem., v. 15, no. 1, p. 161–166.

(17) ——— and Carruth, F. E.
1915. GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED MEAL. *In* Jour. Agr. Research, v. 5, no. 7, p. 261–288. Literature cited, p. 287–288.

(18) ——— ———
1917. IRON AS AN ANTIDOTE TO COTTONSEED MEAL INJURY. *In* Jour. Biol. chem., v. 32, no. 2, p. 245–257, 4 charts.

(19) ——— and Ray, B. J.
1912. A METHOD FOR THE REMOVAL OF THE TOXIC PROPERTIES FROM COTTONSEED MEAL. A PRELIMINARY REPORT. *In* Science, n. s., v. 36, no. 914, p. 31–32.

PLATE 1

Effect of feeding cottonseed feeds to pigs:

A.—Pig 3, showing condition on the ninety-fourth day on a feed containing ether-extracted cottonseed kernels.

B.—Pig 3, showing condition on the fiftieth day.

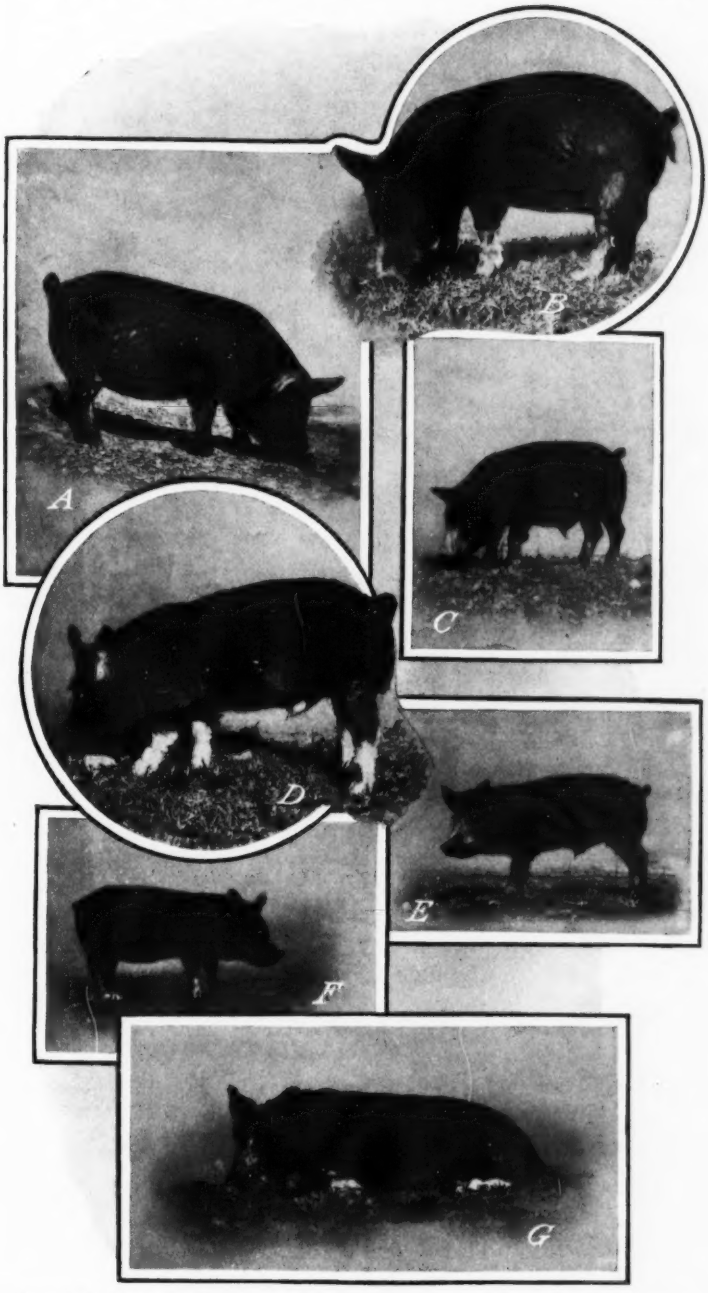
C.—Pig 4, showing condition on the twenty-seventh day on a feed containing vitamines.

D.—Pig 1, showing condition on the fiftieth day on a feed containing cottonseed meal. See figure G.

E.—Pig 2, showing condition on the twenty-seventh day on a feed containing gossypol.

F.—Pig 3, showing condition on the twenty-seventh day on a feed containing ether-extracted cottonseed kernels.

G.—Pig 1, showing condition on the fiftieth day on a feed containing cottonseed meal.


A
B
C
D
E
F
G

FRUIT-FLY PARASITISM IN HAWAII DURING 1916

By C. E. PEMBERTON, *Assistant Entomologist*, and H. F. WILLARD, *Fruit-Fly Quarantine Inspector, Mediterranean Fruit-Fly Investigations, Bureau of Entomology, United States Department of Agriculture*

Since the introduction of parasites of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) into the Territory of Hawaii in 1913, by the Territorial Board of Agriculture and Forestry, more or less continuous notes have been kept, from month to month, indicating the extent of parasitism exerted upon the larvæ of the fruit fly by these parasites and by other species subsequently brought in. Papers presenting summaries of these records, separately for the years 1914 [1] and 1915,[2] have already been published. Opportunity for special investigations of fruit-fly parasites in Hawaii in 1916 has made possible the accumulation of much careful data on fruit-fly parasitism during this year, of much the same nature as that given in the publications just cited, and it is the purpose of this paper to give, possibly more in detail, some results of the work in 1916.

It is felt that a separate record of the conditions of parasitism as existing in Hawaii in 1916, three years after the first and probably the most important of these introductions, will be of no little value and interest to entomologists, by way of comparison with the parasitism in 1914 and 1915, for interesting developments in connection with the question of general parasite introductions and as a necessary contribution to the history of fruit-fly parasitism in Hawaii.

The tabulation during most of the year of the exact degree of infestation of large quantities of host fruits of the fruit fly, from many localities, has been an important part of this work (Tables I–III). All fruits collected for parasitism records on their contained maggots have been accurately counted and placed in separate boxes over sand. The fruit is then kept in the boxes until practically all the contained fruit-fly larvæ have developed, emerged, and entered the sand below for pupation. A record of the total number of larvæ thus developing and pupating is secured. The larvæ quickly pupate after leaving the fruit. The pupæ are placed in vials and later carefully counted in determining the degree to which they have been parasitized. The filing of exact data of this nature from year to year is necessarily the most reliable and positive method of ascertaining the actual degree to which the parasites now

[1] BACK, E. A., and PEMBERTON, C. E. PARASITISM AMONG THE LARVÆ OF THE MEDITERRANEAN FRUIT FLY (C. CAPITATA) IN HAWAII IN 1914. *In* Bien. Rpt. Bd. Comrs. Agr. and Forestry Hawaii, 1913/14, p. 153–161. 1915.

[2] ——— ——— PARASITISM AMONG THE LARVÆ OF THE MEDITERRANEAN FRUIT FLY (C. CAPITATA) IN HAWAII IN 1915. *In* Jour. Econ. Ent., v. 9, no. 2, p. 306–311. 1916.

Journal of Agricultural Research,
Washington, D. C.
la

Vol. XII, No. 2
Jan. 14, 1918
Key No. K—59

established are contributing toward a control of the fruit fly. Such data not only indicate the extent of fruit infestation from various localities, but also the amount of parasitism among the larvæ from month to month and the seasonal efficiency of each parasite.

Seasonal differences in the value and prolificness of certain species of the introduced parasites have been most striking. This is suggested by an examination of any of the parasite notes from almost any locality and by a comparison of emergences of different species for each month of the year. But most convincing proof that seasonal differences exist is obtained by the inspection of records from fruit collected from the same localities month by month. Some species of trees in Hawaii bear fruit, normally a host of the fruit fly, almost continuously throughout the year. The systematic collection of fruit from such trees and the filing of exact data bearing on the extent of parasitism of fruit-fly larvæ secured from such individual trees throughout the year have thrown most light upon the seasonal values of the different parasites.

Mention of possible fluctuations in the abundance of different species has already been made.[1] The work of 1916, wherein it has been possible to concentrate parasites in fruit collections from individual trees, has most impressively shown the rise of the parasite *Diachasma tryoni* Cameron in the summer and fall of the year and its certain decline during the winter and particularly the spring months. Changes in temperature, of no great magnitude, alone seem responsible for this. The parasite *Opius humilis* Silvestri, more hardy and prolific than any of the other introduced species, has been overshadowed by the other species, particularly by *D. tryoni*, and has had its seasonal rise and fall directly the reverse and entirely dependent upon the rise and fall of this species of Diachasma. The slight seasonal changes have little visible effect upon the activities of *Opius humilis*, however, for in the winter and spring, with the decrease in abundance of *D. tryoni*, it rapidly ascends and becomes the most effective check upon the fruit fly (Table III). These interrelations are treated elsewhere by the writers.

The problem of control of the fruit fly in Hawaii through parasites is only partially solved. The four species already established are accomplishing a certain control, particularly in the coffee districts, but a casual survey of the extent of infestation of most host fruits as shown in Table I will convince one of the continued destructiveness of this pest in Hawaii. An average parasitism of 40 per cent of all of the larvæ developing is, numerically considered, of much importance; but from the standpoint of the practical needs of the horticulturist it brings little relief.

[1] BACK, E. A., and PEMBERTON, C. E. PARASITISM AMONG THE LARVÆ OF THE MEDITERRANEAN FRUIT FLY (C. CAPITATA) IN HAWAII IN 1915. *In* Jour. Econ. Ent., v. 9, no. 2, p. 306–311. 1916.

TABLE I.—*Extent of infestation of host fruits by larvæ of Ceratitis capatata in Hawaii during 1916*

Host fruit.	Number of fruits collected.	Number of larvæ of *C. capitata* emerging.	Average number of larvæ per fruit.
Kamani (*Terminalia catappa*)	15,723	149,415	9.5
Mango (*Mangifera indica*)	1,317	2,291	1.7
Coffee (*Coffea arabica*)	41,605	21,224	.5
Strawberry guava (*Psidium cattleianum*)	13,825	22,017	1.6
Black myrobalan (*Terminalia chebula*)	6,615	46,639	7.0
Peach (*Prunus persica*)	669	13,738	20.5
Rose-apple (*Eugenia jambos*)	1,258	7,001	5.5
Chrysophyllum monopyrenum	1,956	4,034	2.0
Brazilian plum (*Eugenia braziliensis*)	4,398	3,808	.8
French cherry (*Eugenia uniflora*)	7,627	6,617	.8
Mimusops elengi	11,883	63,017	5.3
Ochrosia elliptica	77	240	3.1
Kamani (*Calophyllum inophyllum*)	218	737	3.3
Bestill (*Thevetia neriifolia*)	1,532	5,540	3.6
Averrhoa carambola	159	214	1.3
Chinese orange (*Citrus japonica*)	1,588	4,843	3.1
Noronhia emarginata	5,296	9,032	1.7
Guava (*Psidium guajava*)	1,791	12,248	6.8

Table I shows few fruits that have a yearly average infestation of less than two larvæ per fruit. Considering the large quantity of fruits collected during the year, from which the records have been made, an average of two larvæ per fruit is high. It means that great numbers of fruits from all localities are nearly always heavily infested. The fruits collected for such a record are not selected with the purpose of obtaining only heavily infested fruits or only sound fruits. All available fruits that have matured are gathered and brought in whether infested or not. In this manner the exact average condition of fruit-fly abundance, injury, and parasitism is obtained.

To refer again to the tables particular attention should be called to the mango (*Mangifera indica*), guava (*Psidium guajava*), *Mimusops elengi*, *Noronhia emarginata*, and the Chinese orange (*Citrus japonica*). Great numbers of fruit-fly larvæ develop in these fruits and are but slightly parasitized, as shown in the total column for these fruits in Table II. Certain characters of these fruits prevent the parasites from reaching the larvæ within. This in part accounts for the constant presence of this pest, in spite of the establishment of parasites well adapted to the conditions of the country and of great prolificness.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii, 1916*[a]

Host fruit.	Month of collection.	Number of larvæ emerging during first 2 to 6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
Kamani (*Terminalia catappa*)	January	115	22.6	.....	.....	.....	22.6
Do.	March	2,792	30.5	0.6	.....	0.5	31.6
Do.	April	9,558	46.1	1.1	0.1	.03	47.33
Do.	May	1,391	24.7	13.3	.07	.....	38.07
Do.	June	3,094	6.8	16.5	.4	.....	23.7
Do.	July	3,569	2.4	7.3	.9	.1	10.7
Do.	August	4,017	8.4	27.7	.....	.9	37.0
Do.	September	3,526	11.8	53.9	.....	.2	65.9
Do.	October	3,403	15.5	58.5	.4	1.7	76.1
Do.	November	2,299	8.3	51.5	.....	2.3	62.1
Do.	December	1,408	9.0	49.0	.....	1.2	59.2
Mango	June	283	9.5	3.8	6.7	.3	20.3
Do.	July	299	9.7	12.0	3.3	1.3	26.3
Do.	August	47	6.3	4.2	.....	2.1	12.6
Do.	September	53	1.8	5.6	.....	.....	7.4
Do.	October	39	5.1	12.8	.....	.....	17.9
Coffee *b*	February	390	60.7	6.4	.....	.....	67.1
Do.	March	62	4.8	.....	66.1	.....	70.9
Do.	April	1,621	60.3	29.7	4.3	.....	94.3
Do.	May	105	57.1	5.7	.9	.9	64.6
Do.	June	131	85.4	.....	.....	.....	85.4
Do.	August	308	17.2	52.9	9.4	.....	79.5
Do.	October	288	59.0	10.4	12.1	.....	81.5
Do.	November	1,192	22.3	11.3	27.9	.....	61.5
Do.	December	643	12.2	14.6	17.1	.....	43.9
Strawberry guava	April	2,705	38.1	.1	2.7	.1	41.0
Do.	May	48	27.1	.....	.....	.....	27.1
Do.	June	979	10.2	6.7	6.0	.....	22.9
Do.	July	1,013	10.3	46.0	3.5	.4	60.2
Do.	October	189	3.1	14.8	.....	.5	18.4
Do.	November	34	8.8	41.1	5.8	.....	55.7
Black myrobalan	October	3,081	11.2	3.8	.2	.3	15.5
Do.	November	2,518	8.5	8.6	1.0	1.1	19.2
Do.	December	1,319	27.4	5.3	1.8	12.8	47.3
Peach	March	2,311	3.2	.....	.08	.....	3.28
Do.	April	951	15.0	.5	.4	.....	15.9
Rose-apple	May	170	4.1	37.6	2.3	.....	44.0
Do.	June	1,089	2.2	62.4	.1	.....	64.7
Do.	July	14	.....	57.1	.....	.....	57.1
Chrysophyllum monopyrenum	January	996	6.5	1.1	1.5	.....	9.1
Do.	February	702	1.2	.....	.5	.....	1.7
Do.	March	378	1.0	.....	1.0	.....	2.0
Do.	April	78	74.3	.....	12.8	.....	87.1
Do.	May	634	57.0	.....	.9	.....	57.9
Brazilian plum	June	1,306	19.5	16.6	22.3	.....	58.4
Do.	November	78	7.6	16.7	29.4	.....	53.7
Do.	December	33	6.0	3.0	21.2	.....	30.2
French cherry	January	41	34.1	.....	14.6	.....	48.7
Do.	March	95	8.4	.....	.....	.....	8.4
Do.	April	25	64.0	.....	.....	.....	64.0
Do.	May	114	10.5	32.4	9.6	.....	52.5

a Most of the fruits represented in this table were collected about Honolulu at low elevations; the coffee, however, was collected on the island of Hawaii, in addition to localities in Honolulu, and much of it came from points 1,000 to 2,000 feet above sea level.

b The June collection of coffee came from the Waianae Mountains, where only *Opius humilis* was established.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii, 1917*—Con.

Host fruit.	Month of collection.	Number of larvæ emerging during first 2 to 6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
French cherry	June	479	4.8	6.2	11.4	.....	22.4
Do	July	60	1.6	1.6	48.3	.....	51.5
Do	December	862	3.4	10.7	8.4	.....	22.5
Mimusops elengi	January	181	4.4	.....	.5	.....	4.9
Do	February	314	.6	.....	.....	.....	.6
Do	March	976	3.5	.....	.....	.....	3.5
Do	April	10,514	8.4	.....	.01	.02	8.43
Do	May	2,535	10.0	.3	.3	.1	10.7
Ochrosia elliptica	May	103	15.5	36.8	23.3	.....	75.6
Do	June	18	16.6	5.5	22.2	.....	44.3
Kamani (*Calophyllum inophyllum*)	January	819	.8	.....	.2	.2	1.2
Do	March	210	1.9	.....	.....	.....	1.9
Do	April	167	4.7	.....	.....	.....	4.7
Do	December	116	.8	.....	.....	.....	.8
Bestill	March	18	5.5	.....	.....	16.6	22.1
Do	May	22	.....	.....	.....	.....	.0
Do	June	85	7.0	9.4	9.4	.....	25.8
Do	August	173	10.9	3.4	2.3	1.7	18.3
Do	September	274	3.6	2.5	22.6	5.8	34.5
Do	October	392	3.3	3.3	19.1	9.6	35.3
Do	November	84	.....	.....	39.2	7.1	46.3
Do	December	159	1.2	.....	16.9	13.8	31.9
Averrhoa carambola	September	32	40.6	.....	.....	.....	40.6
Do	October	84	13.0	.....	.....	.....	13.0
Chinese orange	January	143	4.1	.....	.7	.....	4.8
Do	March	319	.....	.....	.2	.....	.2
Do	July	39	.....	7.7	5.1	.....	12.8
Do	August	258	3.8	1.5	2.7	.....	8.0
Do	September	76	23.6	.....	.....	.....	23.6
Noronhia emarginata	June	1,767	.2	1.5	.05	.....	1.75
Do	July	853	.4	1.6	.....	.....	2.0
Guava	May	403	3.4	.4	.....	.....	3.8
Do	June	781	2.0	.2	.....	.....	2.2
Do	July	248	2.5	3.6	.4	.....	6.5
Do	September	1,670	5.2	2.1	.05	.....	7.35
Do	October	496	4.6	.....	.....	.....	4.6

The data in Tables I and II covering the guava are of unusual interest. This shrub grows wild and luxuriantly over most of the uncultivated portions of the islands up to an elevation of 1,500 to 2,000 feet, and fruits throughout the year. It is not generally considered by the layman of Hawaii as a favored host of the fruit fly, though of the 18 host fruits given in Table I, it stands fourth in degree of infestation, showing, from 1,791 fruits collected during the year, an average infestation of 6.8 larvæ per fruit. Infestation of this fruit is not easily detected until it has decayed. The larvæ are small and nearly all inconspicuous at the time the fruits are picked and eaten or converted into preserves or jelly. This fruit, though heavily infested in most localities, protects the larvæ

from parasite attack and thus constantly liberates great numbers of flies throughout the year and serves but in a small measure toward the building up of favorable quantities of parasites.

TABLE III.—*Total parasitism by month of all larvæ of Ceratitis capitata collected in Hawaii during 1916*

Month.	Number of larvæ.	Percentage of parasitism.				
		Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
January	2,295	5.5	0.4	1.0	0.08	6.98
February	1,406	17.6	1.7	.2	.....	19.5
March	7,161	13.7	.2	.6	.2	14.7
April	21,619	34.8	2.1	.7	.04	37.64
May	5,525	19.6	6.1	.9	.09	26.69
June	10,013	7.8	15.5	4.5	.009	27.809
July	6,134	3.7	13.0	1.8	.02	18.52
August	4,803	8.8	27.0	.8	.9	37.5
September	5,631	9.7	34.0	1.1	.4	45.2
October	7,972	13.8	27.4	1.7	1.4	44.3
November	6,205	11.0	25.2	6.7	1.4	44.3
December	4,540	13.3	20.9	5.3	4.6	44.1

A comparison of fruit-fly parasitism data secured during the years 1914, 1915, and 1916 would indicate that the parasites now present in the Territory have reached their maximum degree of development and can hardly be expected to attain a greater control of the fruit fly than that evidenced in 1916. There has been some variation during the past three years in the activities of the different species introduced, as already noted in regard to the fluctuations in abundance of *Diachasma tryoni* and *Opius humilis;* but the check upon the work of this pest by the present parasites can hardly exceed its present limits. Some hope, however, is yet felt for the parasite *Tetrastichus giffardianus* Silvestri. It has gradually increased in numbers about Honolulu since its establishment late in 1914. Certain valuable points in its favor may enable it, after further acclimatization and general adaptation to new environment, to exceed the work of the braconids and thus increase the total average parasitism.